600/7-79-211

NBS

U.S. Department of Commerce

National Bureau of Standards

Office of Environmental Measurements
Washington, DC 20234

EPA

United States Environmental Protection Agency

Office of Environmental Engineering and Technology
Washington DC 20460

EPA-600/7-79-211
November 1979

Research and Development

Assessing the Environmental Impact of Fossil-Fuel Based Energy Sources

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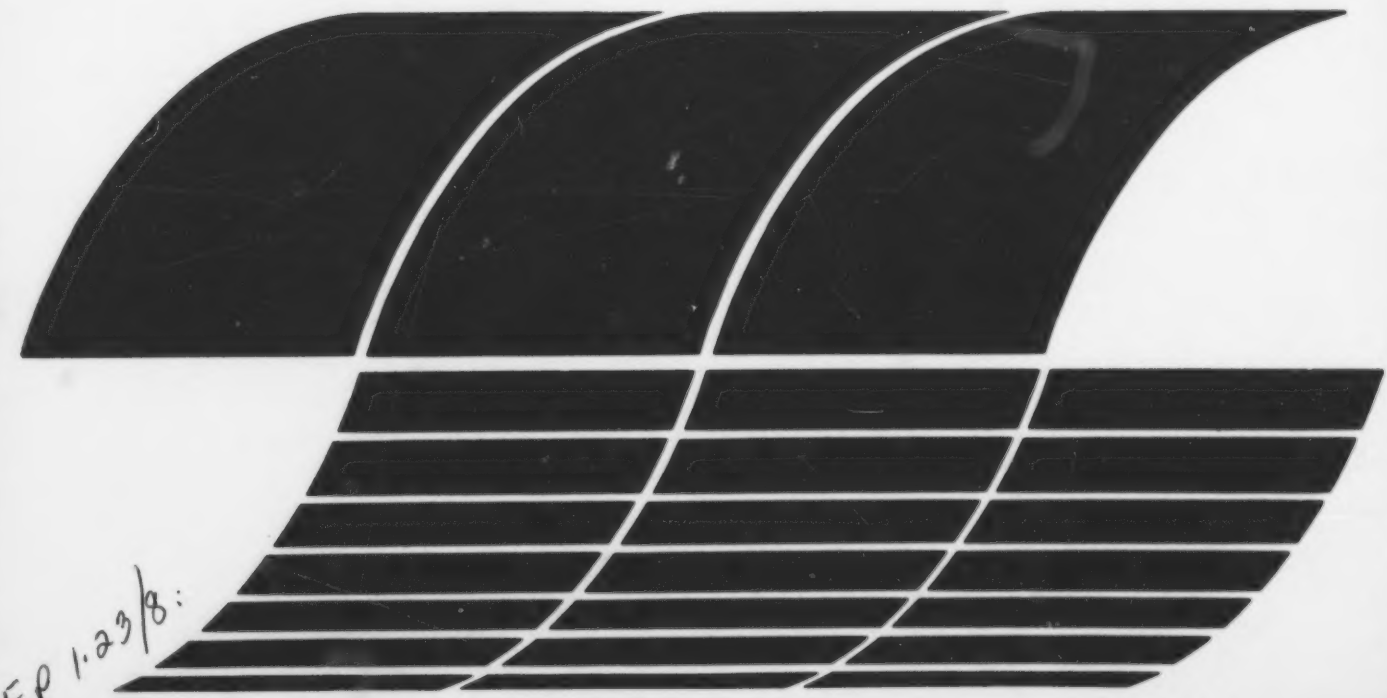

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ASSESSING THE ENVIRONMENTAL IMPACT OF FOSSIL-FUEL BASED ENERGY SOURCES: MEASUREMENT OF ORGANOMETAL SPECIES IN BIOLOGICAL AND WATER SAMPLES USING LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

by

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Environmental Protection Agency
Washington, DC 20460

This study was conducted
as part of the Federal
Interagency Energy/Environment
Research and Development Program

Prepared for

OFFICE OF ENERGY, MINERALS, AND INDUSTRY
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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FOREWORD

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ABSTRACT

A new measurement approach for the determination of trace organometals in energy related environmental samples is described. The method is based on liquid chromatographic separation with electrochemical detection. A detailed description of the development of the electrochemical detection system, optimized for reducible analytes, is given. The conditions for the separation of methyl-, ethyl-, and phenylmercury in under 16 minutes by charge-neutralization reverse-phase chromatography are developed. Also, a separation of a number of organoleads, including trimethyl- and triethyllead, are described. The potential interferences in this type of organometal determination are investigated. Sample preparation methods and improvement in the detector selectivity are described that overcome these interferences. A significant improvement in the selectivity of the detection system has been investigated using a differential pulse waveform.

Sample preparation methods for the determination of methylmercury in biological tissue are examined and measurements are made on two research materials: lyophilized tuna and shark meat. A column preconcentration procedure for methyl- and ethylmercury in natural water samples is developed.

This report was submitted in partial fulfillment of Contract EPA-IGA-D5—E684 by the National Bureau of Standards under the sponsorship of the U.S. Environmental Protection Agency. This report covers work performed during the period of May 1977 to June 1978.

Keywords: Organometal speciation, electrochemical detection in liquid chromatography, methylmercury, trimethyllead, column preconcentration, tissue preparation.

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1. INTRODUCTION

Mounting concern over the presence and role of toxic substances in the environment and living systems resulting from energy generation has created the need for selective and sensitive measurement techniques. Methodology now exists for the analysis of heavy metals (an important class of pollutants) in a variety of matrices, and considerable effort is being devoted to the collection of data on the levels of these elements in biota and water samples. However, some toxic elements can be transformed, by biological and chemical processes, into organometals. Organometallic species have different properties from their inorganic counterparts. Thus, in order to fully understand the role of these toxic materials, it is necessary to measure the exact chemical form of the element in the sample. This research project describes the development of a new analytical technique for the speciation of organometals from fossil-fuel based energy sources.

The measurement technique is based on a separation of several organometal species by liquid chromatography with detection by reductive voltammetry. Primarily, the work described here covers the optimization of the choice of working electrode materials, solvent purification, and applied waveforms. Separations of several organomercury and organolead species are also described.

2. SUMMARY AND CONCLUSION

This report describes the development of a new hybrid detection approach for the measurement of organometal cations, liquid chromatography combined with reductive electrochemical detection (referred to as LCEC). Considerations unique to reductive LCEC are discussed in detail including the choice of working electrode material (gold amalgamated with mercury was selected), purification of the solvent (deoxygenation and cathodic electrolysis), and system construction (dampening of flow pulsations and the need for an inert atmosphere). The waveform applied to the detector cell affects the sensitivity and selectivity. Differential pulse detection has been found to provide the best selectivity while still providing detection limits in the low ppb (μg/g) range for several organometals.

The separation of organomercury cations was accomplished by reverse-phase chromatography with the addition of a strong complexing agent. Organolead cations form only weak complexes, but can be separated directly on a C_{18} column.

The reduction potentials of many organotin, lead and mercury cations, in a chromatographically compatable solvent, are reported.

Methods have been developed for water sample preconcentration and biological tissue preparation for the measurement of organomercury species by LCEC. Methylmercury has been determined in two reference materials.

3. ORGANOMETAL CHEMISTRY

Organometals are becoming recognized as an important class of compounds in many phases of chemistry. Their unique properties make them important in industrial, agricultural, biochemical, and environmental systems. The widespread influence of organometals has created the need for selective and sensitive analytical measurement techniques, which has provided the impetus for this research project.

Inorganic Chemistry of Organometals

Organometallic compounds are those with direct carbon-to-metal (or metalloid) bonds. These many be pi interactions between the metal and alkenes, alkynes, or arenes; or the bonds may be sigma as the alkyl organometals. Only the sigma bond organometals will be considered here as these are the species most investigated for their biological interactions (1).

There are a number of metal and metalloid elements that form waterstable organometallic species including Hg, Pt, Au, Tl, Pb, Sn, Pd, As, Sb, Co, Cr, Te, and Se. However, from an environmental standpoint the compounds of Hg, Pb, Sn, As, and Se are the most important.

The chemical behavior of organometallic compounds is dependent on the central metal element and on the degree of coordination and character of the hydrocarbon groups. Completely alkylated metals are usually volatile liquids at room temperature. The partially, organically coordinated species of Pb, Hg, Sn, and Tl can form dissociated cations in aqueous solution (2). This contrasts the similar compounds of As, Sb, and Se that do not form free cations when dissolved in water. Rather, the organometal-anion bond shows mostly covalent character.

Organometals are generally organophilic in behavior. This is especially true as the organic coordination increases and as the R group length is increased. The formation of strong neutral complexes of the cationic organometals also enhances the organophilicity.

It is possible to have interreaction between an organometal and a metal ion with the transfer of one or more organic groups. For example trimethyltin cation will methylate mercury (II) ion (3):

$$(CH_3)_3Sn^+ + Hg^{++} \rightarrow (CH_3)_2Sn^{++} + CH_3Hg^+$$

This type of reaction is termed "transalkylation".

Most organometal species are subject to hydrolysis in strong acid (4) but are stable to dilute acid and alkaline hydrolysis in some cases (5). Some of the organometals are subject to photolysis with intense U.V. light (3).

It is important to take these characteristics into account when developing an analytical procedure for organometal analysis.

Environmental Importance

Organometallic compounds have been known to have toxic effects on living organisms since the 1800's (1). The organometal compounds of arsenic and mercury were found to have biocidal effects and were therefore used in the treatment of disease. But only recently, it has been discovered that a number of heavy metal (and also metalloid) elements could be transformed in living systems into organometallic compounds. Early studies on these conversions have shown that certain bacteria and fungi can methylate inorganic metal ions (1). Later it was found that this "biological methylation" can also occur in the presence of suitable coenzymes, namely vitamin B_{12} and S-adenosylmethionine (6).

These "biomethylation" reactions are important from two major standpoints. First, the methyl products are generally much more toxic than their inorganic counterparts (1). For example, CH_3Hg^+ is often quoted as being 100 times more toxic than Hg^{++}. The enhanced toxicity may be related to their enhanced lipophilicity and the associated increase in the residence time in the tissues. The toxicity is further complicated by the tendency of organometals to be "bioaccumulated" throughout the food chain of a living ecosystem. For example, in a marine environment, the lower forms of life such as plankton accumulate mercury to over 1,000 fold that found in the surrounding seawater (7). Much of the mercury is retained as life progresses up the food chain. Since humans are often at the top of the food chain, the levels of organometals are very important. For example, the outbreak of "Minimata disease" in Japan was a result of high methylmercury levels in fish (caught in mercury polluted water).

The second major influence of the biomethylation reactions is the change in the mobility of toxic elements in the environmental systems. Often the transport of heavy metals is facilitated by the formation of soluble and/or volatile species. Totally coordinated species such as $(CH_3)_2Hg$ and $(CH_3)_3As$, and even complexes of the cationic organometals are sufficiently volatile for atmospheric transport far from sources of contamination.

Another very important source of a wide variety of organometals in the environment is waste from their industrial and agricultural use. Organo-arsenic, mercury and lead compounds are widely used as fungicides and pesticides (1). Organotins are used in the polymer industry as catalysts and in antifouling boat paint. Totally methylated species such as $(CH_3)_2Cd$, $(CH_3)_2Hg$, and $(CH_3)_3As$ are used in electronics manufacture to deposit small amounts of pure metals. And of course, tetraethyllead is an important gasoline additive.

In order to evaluate the environmental impact of organometals, both naturally formed and synthetically produced, selective and very sensitive analytical techniques are needed (6,8). Methods must be developed that can "speciate" the different forms a metal may take in a sample.

4. ANALYTICAL MEASUREMENT OF ORGANOMETALS

There are many approaches to the speciation of organometals. In all cases there is a selective separation (either chemical or chromatographic) and subsequent selective detection.

Perhaps the most rapid separation approach for the volatile organometals (or their derivatives) is gas chromatography. This analytical technique is in an advanced stage of development, with a wide variety of commercially-available high-performance column materials. This approach depends heavily on the volatility of organometal species. For totally coordinated species such as $(CH_3)_2Hg$ (B.P. 96 °C) and $(CH_3)_4Pb$ (B.P. 110 °C), and for volatile derivatives of the cationic organometals such as CH_3HgCl (B.P. 170 °C) (9), there is no difficulty using this separation technique. However, for many other organometals, easily volatile and thermally stable derivatives are not available, limiting the applicability.

There are four major detection approaches for organometal analysis by gas chromatography. The following table summarizes them:

TABLE 1

Technique	Principle	Sensitivity	Selectivity
electron capture	analyte absorbs β particle from Ni^{63} source and ions formed are collected at an anode	100 pg (10) 20 pg (11)	fairly selective but also responds to halide, nitro, and sulfur compounds

(Continued)

TABLE 1 (Continued)

Technique	Principle	Sensitivity	Selectivity
mass spectrometry	low pressure analyte gas is bombarded with electrons, resultant positive ions are accelerated and separated by a magnetic field, ions collected at a cathode; mass-to-charge ratio may be scanned or a single mass may be monitored	500 pg (12, 13)	very selective but scanned spectrum may be cluttered with interferent's ions
flameless atomic absorption	analyte is atomized in a continuously heated electrothermal graphite furnace and absorption of atom resonant line is measured	100 pg (14) 10 ng (15)	extremely high element selectivity but no differentiation of species of the same element
microwave cavity emission	analytes are introduced into inert gas plasma generated by microwave absorption, emission by excited atoms is monitored	0.5 pg (16) 10 pg (17)	very high element selectivity

The electron capture detector is presently the most widely used in organometal analysis because of its simplicity and sensitivity. However, the selectivity is relatively poor and therefore rather extensive sample cleanup is required before analysis with this detection approach. For example, when analyzing solid homogenates for methylmercury, it is first necessary to form the chloride in 1 M HCl and then extract into toluene. Then the toluene layer is back extracted into basic, aqueous cysteine solution and subsequently extracted after reacidification with HCl into toluene (5). This cleanup is necessary to remove interfering sample constituents. This laborious preparation procedure enhances the possibility of systematic error and lowers recovery of CH_3Hg^+ to between 80 and 90 percent. The selectivity of mass spectrometry and atomic absorption is much higher, but the apparatus is expensive and complex. Also the sensitivity is relatively poor and often extensive sample preconcentration is needed prior to analysis (12).

Another separation technique that only deserves a brief mention in organometal speciation is thin layer chromatography. After a separation the "spots" are either identified colorimetrically (18) or analyzed by another technique such as A.A. (19). This approach is adequate when the character of the samples is well known (as in quality control) and the amounts of organometals are fairly high (i.e., mg/g).

In special cases, selective chemical reactions can be used to differentiate between the organometallic and inorganic forms of an element. For example, in one such approach volatile CH_3HgCl is formed and distilled over (Hg^{++} is not volatile under these conditions). The CH_3HgCl is then reduced to Hg° and measured by cold vapor atomic absorption (20). Selective extraction has been used to differentiate organoleads (21). Radiochemical assay can be used with selective chemistry for the mercury species as (surprisingly) exchange of radioactively labeled Hg^{++} is rapid with CH_3Hg^{+} in the sample (22). However, the scope of this approach is limited to differentiation between organic and inorganic forms of only one metallic element, and cannot be used to investigate new, or unexpected species in a sample.

Some work has been done recently using liquid chromatography as the separation approach followed by selective detection. This approach has a fundamental advantage over gas chromatography, in that the analytes do not have to be volatile. Therefore, the requirement of forming volatile, thermally stable derivatives does not apply. The problem with liquid chromatography is finding a suitable detection system for the job. Present work in analyzing organometals is limited to the use of atomic absorption detection except for some work using atomic fluorescence by Van Loon (23).

Perhaps the primary consideration in liquid chromatography with atomic absorption detection (LCAA) is the interface of the LC solvent output to the final formation of free atoms. An easily interfaced atom reservoir is the premix flame (24). The flow rates used in HPLC (0.5 to 2 mL/minute) are just about right for the burner uptake rate (1-3 mL/minute). However, the flame as an atom reservoir is not very sensitive, perhaps a few ppb can be detected for good elements (without accounting for the dilution in the LC).

A somewhat more sensitive atom reservoir is the electrothermally heated graphite furnace. This device may be used in a continuous mode if the sample stream is split (25). However, in using this approach most of the LC effluent is wasted, increasing the detection limit. In another approach, a small (about 100 µL) sample can be taken of the effluent periodically, for subsequent analysis (26). In the discontinuous mode of operation an autosampler takes a sample of the effluent every 50 seconds, dries it in the furnace, and subsequently atomizes the sample. However, this sample rate is too slow to maintain the fidelity of a high efficiency separation and thus resolution and detection limits suffer greatly (26) unless the chromatography is run very slowly.

Perhaps the most serious drawback of LCAA is the poor limit of detection. Although graphite furnace atomic absorption is quite sensitive for some elements (such as Pb), it is relatively poor for many elements that form

interesting organometallic species such as As, Sb, Hg, Sn, Se, and Te. It must be remembered that the HPLC separation generally dilutes a 20 µL sample to 0.2 to 2 mL losing one to two orders of magnitude in detection limits for any detection system.

A second serious problem unique to organometal analysis by GFAA is that the analytes are very volatile species and it is difficult to atomize them without great losses by direct volatilization as neutral compounds (which are not detected). In the autosampler mode, the desolvation and ashing temperatures are kept quite low and only the atomization temperature is high (26). However, under these conditions it is absolutely necessary to use background correction, as nonspecific absorption of other sample constituents is very likely without a pre-ashing cycle. This is a disadvantage as the use of continuum background correction generally lowers the sensitivity somewhat. This nonspecific absorption problem becomes quite severe when solvents with greater than 0.1 M ionic content are used for the chromatography. This severely limits the applicability of this detection approach in ion exchange.

Although the LCAA approach provides very high element specificity (in the background corrected mode of operation) it does not discriminate between different species of the same element if they coelute in the chromatography. Worse yet there is no way to gain further qualitative information on a suspected analyte peak (besides the element content). It is necessary to use a second detection approach to confirm the absolute identity of the analyte species in a completely unknown sample.

Clearly, a more sensitive and reliable detection technique is needed for the measurement of organometals separated by liquid chromatography. This report of investigation describes the development of such a detection system.

5. EXPERIMENTAL PROCEDURES

DEVELOPMENT OF LCEC

Applicability

A promising recent development in the separation and measurement of complex samples is liquid chromatography with electrochemical detection (LCEC). In this approach an amperometric (or coulometric) flow cell detector is used to monitor components separated by HPLC, the selectivity is provided by the chromatography and from the use of the electrochemical cell. This analytical approach shows promise in the measurement of trace organometals since many of these species show electroactivity (27,28).

Historical Development

LCEC got its start in the early 1950's with some work done by Kemula (29) using D.C. polarography coupled to ion exchange chromatography. He termed the new approach "chromato-polarography" a name that has given way to LCEC. Most of the applications of this technique before 1970 employed amperometry at a DME (30-52). Only reducible species were detected.

Perhaps the most significant development in LCEC was the work done by Ralph Adams and Peter Kissinger (53). In this new approach a solid electrode, carbon paste was used for the working electrode material. This gave a potential range primarily suited for the oxidation of organic compounds. Since this development many papers have been published by Kissinger and his associates on the application of LCEC to environmental and clinical analysis. In the short span of five years or so, LCEC has become an important tool for the selective analysis of complex mixtures.

Basic Principles

The electrochemical detector for liquid chromatography consists of a low volume (typically 1 to 100 μL capacity) flow cell containing a working, auxillary, and reference electrode. There are basically three methods of providing working electrode contact with the flowing stream. The solution can be passed over a flat electrode giving a laminar flow (53), or it can impinge directly with radial flow (52), or it can flow through a tubular (often with a packed bed of electrode material) electrode which is particularly useful in coulometric detection (54-56). In all cases, the solution flow decreases the thickness of the Nernst layer through which the analyte

species must diffuse, increasing the faradaic current relative to quiescent conditions. When amperometry is used, the faradaic current is proportional to the flow rate to the 1/2 to 1/3 power (57).

The sensitivity of the electrochemical detector depends on the ratio of the analyte faradaic current to the background noise currents. In constant potential amperometric detection the limiting background noise arises from flow fluctuations in the faradaic current (generated by electroactive impurities in the solvent or the solvent's decomposition). Therefore, to minimize noise levels in the amperometric mode, the fluctuations in solution flow must be minimized by pulse dampening of the chromatographic pumping system and by good hydrodynamic design of the detector cell to minimize turbulence. Also the magnitude of the unwanted solvent faradaic current should be minimized, since the noise is directly proportional to this quantity (58). Coulometric detectors are less flow sensitive since all of the analyte is converted in the electrochemical process and this is the quantity measured. However, the background faradaic current at the large electrode (needed to achieve 100 percent conversion of the analyte) does have a flow dependence and ultimately limits the sensitivity (usually a factor of 10 poorer than amperometry at the same potential). The differential pulse mode has very low flow dependence (58) and thus flow fluctuations would not produce the limiting noise except in situations where either the flow pulsations or the background current were exceedingly large. Rather, the sensitivity limitation is a result of capacitative charging currents generated by the pulsed potential. The following table compares these three waveforms used in LCEC:

TABLE 2

Waveform	Sensitivity in LCEC (mol/L)	Selectivity	Advantages/Limitations
amperometric	5×10^{-9}	fairly low, senses all electroactive species below applied potential	simple, inexpensive, and sensitive; however, it is flow dependent and not very selective
coulometric	1×10^{-7}	fairly low, as above	absolute calibration of the coulomb, low flow dependence; however, it has a complex electrode
differential pulse	1×10^{-8}	very high, senses only electroactive species with $E_{1/2}$ within pulse height (5-100 mV)	high selectivity, low flow dependence, but instrument required is more complex

Another important aspect in the application of LCEC is the choice of working electrode material, since this determines the useable potential range. The following table lists some electrode materials used in LCEC work:

TABLE 3

Material	Potential Range in V, pH 4	Advantages/Limitations	Application
carbon paste	+1.0 to 0.0 (53)	easily resurfaced	oxidations only
DME	+0.1 to -1.5 (50)	continuously resurfaced, large charging current	reductions only
glassy carbon	+1.0 to -1.0 (59)	difficult to resurface	oxidations and reductions
mercury coated platinum	-0.1 to -1.1 (60)	resurfaceable	reductions only
WIGE[a]	+1.2 to -1.2 (61)	resurfaceable	oxidations and reductions
platinum	+0.90 to -0.5 (61)	formation of surface oxides, difficult to resurface	oxidations only
GAME[b]	-0.1 to -1.2 (28)	easily resurfaced	reductions only

[a] WIGE: Wax Impregnated Graphite Electrode.

[b] GAME: Gold Amalgamated Mercury Electrode.

Applications

LCEC can be applied to a wide variety of electroactive analytes. Table 4 lists some of the classes of compounds that would be readily amenable to electrochemical detection.

TABLE 4. APPLICABILITY OF LCEC

Oxidations	Reductions
Hydroxylated Aromatics	Heavy Metal Ions
Quinolines	Nitro Compounds
Catecholamines	Diazo Compounds
Amides	Organometals
Amines	Oxidations at Hg
Mercaptans	Halides
	Sulfide, Cyanide

Possible Interferences

The great potential of LCEC for analyzing complex mixtures lies in the selectivity achieved from the liquid chromatographic separation and from the electrochemical detection. However, LCEC can suffer interferences from sample constituents. Electroactive species that coelute with the analyte can be the cause of a positive additive interference, thus, it is incumbent on the sample preparation to eliminate this type of interference. Although nonelectroactive species give no response at the detector electrode, the presence of excessive amounts of strongly adsorbed (non-analyte) species that coelute with the analyte could passivate the electrode. This will decrease the detector response to the analyte causing a negative, multiplicative interference. Finally, substances such as complexing agents that bind the analyte so that it changes its behavior in the chromatographic system will mask the analyte signal.

As in any analytical method, it is important to be aware of, and to avoid potential interferences in the analysis scheme, right from sample collection to the final measurement step.

ELECTROACTIVITY OF ORGANOMETALS

Cyclic Voltammetry

In order to develop a LCEC method it is necessary to first investigate the electroactivity of the analyte species. One rapid and simple method of obtaining qualitative information about the redox characteristics of analytes

is to use cyclic voltammetry. To begin this work, cyclic voltammograms were run on many potentially interesting organometals in a solvent thought appropriate for the chromatography. The potential range of -0.1 to -1.4 V (versus the detector's Ag/AgCl 3 M Cl^- reference electrode) was scanned for a 10^{-4} M solution of the organometal in a solution that was 50 percent MeOH/water, 0.05 M in NH_4OAc, pH 5.5. This electrolyte was chosen to aid the solubility of the organometals (with MeOH), discourage the formation of OH^- complexes (pH 5.5), and provide only a weakly complexing anion (acetate). The GAME was the working electrode and was prepared as described in the section comparing electrode materials (vide infra). The peak potentials E_p for the first reduction wave are tabulated in Table 5.

TABLE 5

Compound	E_p (in V)	Compound	E_p (in V)
CH_3Hg^+	-0.35	$(CH_3)_3Sb^{++}$	-0.94
$CH_3CH_2Hg^+$	-0.33	$(C_6H_5)_3Sb^{++}$	-0.77
$C_6H_5Hg^+$	-0.65	$CH_3(C_6H_5)_3Sb^+$	-0.80
$(pOH\text{-}C_6H_4)Hg^+$	-0.20	$(C_6H_5)_4Sb^+$	-0.89
CH_3Sn^{+++}	-1.07	$(CH_3)_3Pb^+$	-0.57
$(CH_3)_2Sn^{+++}$	-0.87	$(CH_3CH_2)_3Pb^+$	-0.77
$(CH_3)_3Sn^+$	-0.90	$(C_6H_5)_3Pb^+$	-0.50
$(C_6H_5)_3As$	-0.45	$(CH_3CH_2)_2Pb^{++}$	-0.78
$(CH_3)_2TeI_2$	-0.1	$(C_6H_5)_2Pb^{++}$	-0.65
$(nBu)_3Sn^+$	-0.93	$(C_6H_5)_2Ti^+$	-0.44

In the reduction of most of the organometals tested, the first reduction occurs at potentials lower than -1.0 V and therefore would be readily amenable to LCEC analysis.

Possible Reduction Mechanisms

Much electrochemical research has gone into the elucidation of the reduction/oxidation mechanisms of organometals (27). In many cases the

monocationic organometal species are reduced with one electron per molecule to a free radical:

$$Et_3Pb^+ + e \rightarrow Et_3Pb\cdot \rightarrow Et_6Pb_2 \quad (27)$$

$$CH_3Hg^+ + e \rightarrow CH_3Hg\cdot \rightarrow (CH_3Hg)_2 \rightarrow (CH_3)_2Hg + Hg^0 \quad (27,62)$$

$$(CH_3)_3Sn^+ + e \rightarrow (CH_3)_3Sn\cdot \rightarrow (CH_3)_6Sn_2 \quad (27)$$

Seldom are the first reduction products the metals themselves. This is in contrast to the reduction of many free metal ions. The formation of soluble products as above is an advantage in LCEC analysis, particularly at high analyte concentrations where the reduction product (if insoluble) would coat the solid electrode causing passivation. Thus the electrochemical detection approach is elegantly suited to the measurement of organometals.

LCEC SYSTEM FOR REDUCTIONS

Construction Considerations

In order to develop an LCEC system that would have the high selectivity necessary for the measurement of organometals in environmental and biological samples, it was necessary to improve on the past work done on LCEC for reductions. Early work on reducible species used the DME, with the separated solution impinging on the electrode (40). The continual growing and falling of the mercury electrode gives rise to changing capacitative and faradiac currents. The current fluctuates with the electrode size so that the chromatographic readout had a rather annoying oscillating baseline, unless heavily damped. In addition, the sensitivity of DME amperometry is rather poor (10^{-6} mol/L). The DME detectors were inconvenient because the capillaries used to deliver the mercury drops are easily clogged due to impurities deposited at the tip. Later workers investigated the use of single mercury drops, in one application a hanging mercury drop electrode was used (51) and in another a tube filled with mercury served as the working electrode (63). Also mercury coated platinum was used (51,60) as an electrode with better hydrodynamics than the spherical mercury drops.

In order to select the optimum electrode for reductive LCEC several considerations were taken into account: the cathodic potential limit, electrode surface homogeneity, ease of refinishing, and reproducibility. As a test electroactive organometal, the methylmercury cation was chosen because its redox characteristics were well investigated (64) and the first reduction step was found to be somewhat reversible at the HMDE. For each electrode material, a qualitative judgment of the reversibility of this reduction was made since this reflects the ease of the electrochemical process at the electrode surface and to some extent, the reproducibility in analytical measurements.

The electrode materials were evaluated by recording cyclic voltammograms of a 5×10^{-4} mol/L solution of CH_3Hg^+ in a 1.0×10^{-2} mol/L $NaClO_4$

medium with the pH adjusted (unbuffered) to approximately 2.8, 5.0, and 8.0. The reduced data is given in the following table:

TABLE 6

Material	Cathodic Limit[a] (pH 5)	CH_3Hg^+ reduction	Residual Current	Advantages/Limitations
Carbon paste	-0.6 V	irreversible	low	too limited a cathodic potential
Hg-coated carbon paste	-1.0 V	quasi-reversible	high	too high residual current, irreproducible
gold wire	-1.0 V	quasi-reversible	high	residual current high
Hg-coated gold wire	-1.1 V	quasi-reversible	low	low residual current, easily refinished
glassy carbon	-1.2 V	poorly defined	low	poor reduction current for CH_3Hg^+
Hg-coated glassy carbon	-1.0 V	quasi-reversible	low	irreproducible

[a] Potential at which H^+ reduction just began.

These materials were chosen by examination of an excellent text on solid electrode voltammetry by Adams (65). The materials selected had the best cathodic potential ranges. The idea of mercury coating was to extend the potential necessary for the H^+ reduction, as a result of the hydrogen reduction overvoltage on mercury. Gold was selected as a metallic substrate, over platinum, because of the higher overvoltage of gold amalgam (0.80 V) relative to platinum amalgam (0.10 V) (66).

The electrodes tested were prepared in the following manner. The gold electrode was prepared by press-fitting a 1.0 mm diameter, high-purity gold wire into an undersized hole in a ½" teflon rod, exposing about 4 mm of length. Electrical contact was made by drilling a duct to the wire and filling with mercury, with a copper contact wire. The mercury-coated gold electrode was prepared in a similar manner as above except an amalgam layer was prepared by dipping the electrode into a pool of mercury for about 15 minutes. Excess mercury was then wiped off with a Kimwipe. It was necessary

to hold the electrode at a potential of -1.5 V for about 5 minutes in solution to reduce mercuric oxide impurities before the electrode could be used. The glassy carbon electrode consisted of a 6 mm glassy carbon rod epoxied into a machined plexiglas holder with a mercury contact. The face of the electrode had been polished smooth in a glass shop. The carbon paste electrode was prepared from a 1/2" teflon rod that had a well contacted by a threaded brass rod. The well was 6 mm across and was packed with commercial carbon paste (pasted with wax). The brass rod was slotted so that a screwdriver could drive it inward forcing some paste out of the well. This made it exceedingly easy to resurface the carbon paste electrode. A small amount of paste was extruded, then the electrode was rubbed over glossy computer cards to obtain a smooth surface. The electrical contact to the brass rod was made by mercury and a copper lead wire. The mercury coating on the carbon paste and glassy carbon electrodes had to be prepared by electrolysis of a 10^{-3} M solution of Hg^{++} with stirring for 3 minutes at -2.0 V. This gave a dull gray appearance to the electrode. The electrode was then rinsed and transferred to the CH_3Hg^{+} solution for use.

The initial electrode comparison indicated that the mercury-coated glassy carbon electrode (MCGCE) and the gold amalgamated with mercury (GAME) should be further compared using some other test organometals as well as CH_3Hg^{+}. Performance criteria that were considered were residual currents at pH 2.9 and 8.0, as well as the cyclic voltammetry of CH_3Hg^{+}, $(CH_3)_3Sb^{++}$, and $(CH_3)_3Sn^{+}$ at pH 2.9, 5.5, and 8.0. However, the final decision to use the GAME was a more pragmatic consideration of the reproducibility of analytical measurements. It was quite difficult to provide a reproducible mercury coating on the glassy carbon, even when the surface had been cleaned by mild mechanical polishing. The thin mercury layer gave variable electrochemical results. The GAME, however, was simple to refinish and gave reproducible results. The extra thick, liquid mercury surface seemed much less affected by repeated use, where the MCGCE response changed rapidly with use (probably due to electrodeposited impurities on the thin mercury surface). Since the reproducibility and longevity are important considerations in choosing an analytical working electrode in LCEC, the GAME was selected for use.

In order to use the GAME in LCEC, an electrode holder was constructed from a plexiglass electrode blank. A slightly oversized hole was drilled and the gold wire (1 mm diameter) was epoxied into place. The electrode was then polished flat with wet, #600 emery paper and finally with gem polish type A on a wet felt cloth. This gave a shiny, hydrodynamically flat surface. The gold was then amalgamated by soaking for about 5 minutes in mercury. Excess mercury was wiped off with a Kimwipe but enough was left to form a slightly convex mercury surface. When put into use a very cathodic potential (i.e., -1.0 V) is applied to reduce surface impurities. When in continuous use for more than two hours an anodic potential (-0.2 V) was applied for about 5 minutes to oxidize and/or desorb accumulated coatings. After a period of about one day's use, the electrode must be repolished and coated with mercury since excessive impurities accumulate.

The electrode holder is separated from the cell top, which incorporates the reference and auxillary electrodes, by means of a 5 mm thick teflon gasket. This arrangement is shown in figure 1. The basic design is that of Peter Kissinger (available from Bioanalytical Systems) except that the reference and auxillary electrode holder has been modified so that they are less than 1 cm from the working electrode. This configuration was constructed from a Teflon adapter (1/2" normal pipe thread to 1/4") with the reference electrode (isolated by a porous vycor frit) and auxillary electrode (1/16" stainless steel tubing with end fitting) press fit in place. This design was superior to the commercial design for two reasons. First, the solution resistance between electrodes was lowered since the distance is now about 1 cm rather than 3 cm. This lowers the effective time constant of the series solution resistance and electrode capacitance, improving response in the pulsed mode of operation. Also, the dead volume of the entire cell is now about 100 µL rather than about 1000 µL, making it possible to collect fractions, retaining their chromatographic integrity, for subsequent "off-line" analysis. The dead volume to the working electrode remains at about 1 µL.

The amperometric and differential pulse detection was accomplished using a Princeton Applied Research Model 174 which provides a potential range of ±3 V, choice of pulse heights (5 to 100 mV) and times (0.5 to 5 seconds), and current sensitivity as high as 20 nA/10 V.

The system was designed to maintain ambient electrical noises at a minimum since high amplification of very small signals is inherent in the detection approach. To do this, the chromatograph was mounted on an aluminum base plate which was grounded. Aluminum screen and rods were used to fabricate a demountable faraday cage. Power line spikes (frequent at NBS) going into the voltammetric detector supplies were reduced by means of a "glich-free" power supply constructed from an isolation transformer and parallel, bipolar capacitors.

Another important consideration unique to LCEC in a reductive mode, is the necessity to remove oxygen from the system. This is a requirement since oxygen is easily reduced in two stages:

at around pH 5: $O_2 + 2e + 2H^+ \rightarrow H_2O_2$ $\quad E_{1/2} = -0.1$ V

$H_2O_2 + 2e + 2H^+ \rightarrow 2H_2O$ $\quad E_{1/2} = -0.9$ V

These processes will give a response at the detector (when held at potentials greater than -0.1 V) that will contribute to the background current and hence noise. To reduce the O_2 to a minimum, the solvent reservoir must be continuously purged with high purity inert gas such as Linde oxygen-free nitrogen (where O_2 is < 0.5 ppm).

When the chromatograph was constructed with functional detector cell and purged solvent reservoir, a disconcertingly large background current was still obtained. The chromatographic system must have been allowing oxygen to reenter the solvent before it reached the detector. This seemed unlikely at first because most of the system was constructed from stainless

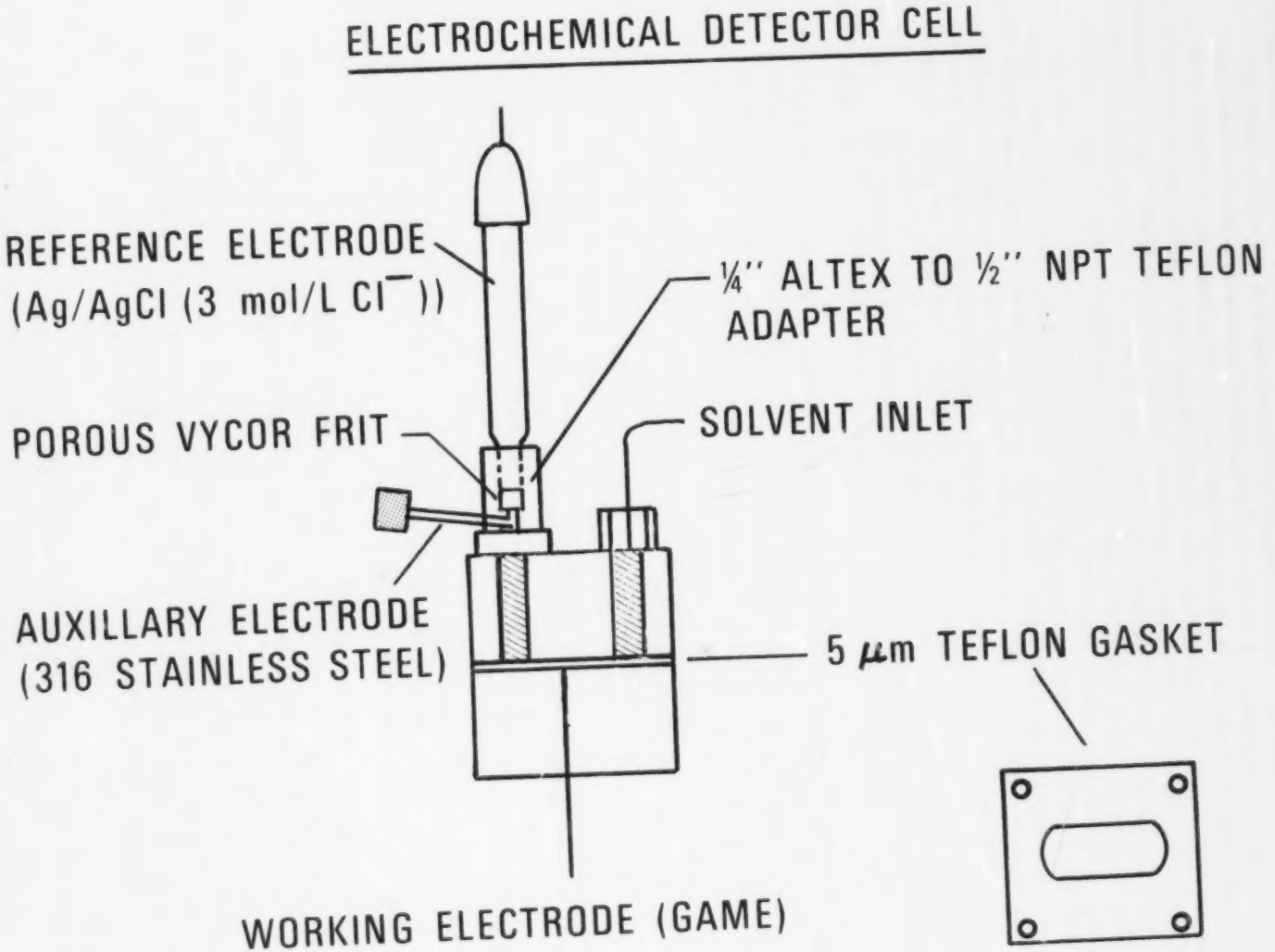


Figure 1. Thin layer electrochemical flow cell.

steel components which are non-permeable to oxygen. However, when the pump was isolated in a plastic garbage bag filled with nitrogen, the excessive background current dropped. The Kel-F fittings in the pump were apparently quite permeable to oxygen. Actually this is of no great surprise as perfluoroalkanes (of which Kel-F is a polymer) are such good solvents for oxygen they have been considered as whole blood replacements. At any rate, because there were teflon-type fittings in the pump, sampling valve, some of the connections, and in the cell spacer gasket, it was decided to enclose the entire chromatographic system within a nitrogen-purged plexiglass box. The enclosure was made 40 x 40 x 100 cm with a large hinged door with a rubber seal and eight threaded latches. As many external controls as possible were incorporated into the box to avoid the necessity of opening the door for trivialities. Provisions that were included are external solvent reservoir filling and draining tubes, two reservoirs with a solvent switch, pump on/off and flow rate controls, external sampling valve switch and syringe, and low volume solvent output switch from the detector to waste (for off-line analysis) or to recycle to the reservoir; all of which were carefully sealed with silicone rubber. The nitrogen flow to the box is regulated by a Whitey valve and measured with a calibrated Matheson, ball-type flowmeter. The oxygen level in the box is continuously monitored by means of a Beckman Oxygen meter with a Clark-type amperometric detector used directly in the gas phase. It is possible to maintain the O_2 level at less than 0.1 percent with a flow of 50 ml/minute of NBS prepurified nitrogen. A small, variac controlled muffin-fan is used to help maintain steady state equilibrium inside the box, especially when filling with N_2.

Other reducible species, especially trace metals, are important to consider in constructing an LCEC system for reductions. It is necessary to maintain reducible species at a minimum to decrease detector background current and noise, and to protect the electrode material from accumulation of reduced (i.e., solid metal or amalgam) deposits. To minimize impurities the solvent is prepared from high-purity reagents (such as Merck Suprapur acetic acid and ammonium hydroxide) and solvents (such as Burdick and Jackson "distilled in glass" methanol). Once prepared the chromatographic solvent was further purified by electrolysis at a mercury cathode (Environmental Science Associates model 214 PM) held at about -1.3 V versus Ag/AgCl. In fact,the electrolysis cell also served as the solvent reservoir in the chromatographic system so that contamination during transfer of the solvent could be eliminated. This also allowed continuous system operation by means of a solvent recycle valve, where normally wasted solvent could be returned for electrolytic purification. When methanolic (or especially those with mercaptans) solvents are used, it is necessary to discard the solvent after 3-4 weeks use as oxidation products in the electrolysis cell (occurring at the platinum counter electrode) begin to accumulate and degrade chromatographic performance.

The chromatographic system is primarily constructed of type 316 stainless steel, used to withstand the high pressures (500-2000 psi) required for high performance columns. At first there was concern that the stainless would corrode (especially in solvents containing complexing agents) releasing electroreducible metal ions that would cause excessive background current.

An examination of the literature (67) indicated that type 316 stainless is about 17 percent Ni, 12 percent Cr, 2 percent Mn, and roughly 60 percent Fe. Fortunately most of the expected ions of these major constituents (Fe^{+2}, Cr^{+3}, Ni^{+2}, Mn^{+2}) are not reducible at potentials below about -1.1 V (68). This still gives a potential range suitable for most organometal analyses. The formation of metal ions from the system can be discouraged by using teflon-lined stainless steel tubing where possible and to employ only type 316 stainless to avoid possible electrolytic corrosion. The presence of these metal ions coming from the stainless is possibly evidenced when complexing solvents are used, by a depression of the electrolytic purification cell potential when the solvent is recycled (indicating excessive reduction at about -1.1 V) and by large initial oxidation currents observed when the working electrode is put at more positive potentials (i.e., -0.4 V) after several days at -1.0 V. Thus, only very small amounts of complexing agents could be used with the stainless steel system. It would be interesting to see how much cathodic potential range (ultimately limited by solvent decomposition) could be gained by constructing an all teflon system. Unfortunately, these systems are limited to 500 psi maximum, putting some constraints on the efficiency of the column packing materials (because larger particles must be used, usually 37-40 μm rather than 5-10 μm).

Another, often important, consideration in high sensitivity—high performance liquid chromatographic detection is pulse dampening of the solvent delivery system. This is particularly important in a flow sensitive detection such as amperometric, where the noise of the detector (and hence sensitivity) is linearly related to flow fluctuations (58). For our system we chose a relatively inexpensive chromatographic metering pump (Altex model 110) that provides some pulse compensation in a novel way. In this pump the single piston is driven by an eccentric cam, where the pump output stroke is very long, but the refill cycle is short (200 msec). Thus the flow is steady for ∿90 percent of the cycle. The remaining pulsations were dampened by means of a nitrogen gas-filled hydraulic dampener, consisting of a capped, 60 cm vertical length of 1/4" stainless tubing joined through a tee between the pump and sampling valve. The gas becomes more compressed during the forward pumping stroke when the pressure is highest. During the pump refill phase decompression of the gas forces stored solvent out of the dampener completing the cycle. This hydraulic dampening provides virtually pulse-free operation at pressures of 500-2000 psi. The disadvantages are the large holdup volume (∿5 ml) of the dampener which precludes its use in gradient elution (as the change in solvent composition is slowed), and the need to periodically replace the gas in the tube that is lost due to dissolution in the liquid under the pressures used. About once weekly, the cap of the dampener tube is removed and the liquid is emptied with suction using a flexible piece of 1/16" teflon tube. Then the tube is connected to a high purity nitrogen supply and the dampener tube is purged and recapped.

The sampling valve chosen was a Rheodyne fixed volume loop type with choice of a 20 μL or 100 μL loop. Throughout this work the 20 μL loop was used as it gave the best efficiency and resolution. The 100 μL loop was tried in order to increase the concentration detection limit, however, the larger loop caused too much loss in resolution particularly of early eluting

components. It was necessary to deoxygenate the samples before analysis by LCEC in the reductive mode. On all columns O_2 showed some retention ($k' \cong 2$) and gave a rather large response since it is about 10^{-3} M in saturated aqueous solutions. The samples are purged with solvent-presaturated, high-purity nitrogen, while connected to the sample loop intake. Several sample container materials were tried. Teflon was found to release too much "stored" oxygen. Plexiglas was better in that respect but adsorbed the organometals readily causing losses at low levels and cross contamination of solutions. Finally, 5-ml pyrex sample vials with polyethylene caps were chosen. Three 1/16" holes in the caps allowed for gas entry and exit, and sample loop intake. These sampling cells showed no significant adsorption and were simple and inexpensive. With this sampling system about three replicates could be obtained from a one mL sample with good reproducibility. One recent suggestion (69) worthy of future investigation is to turn around the problem of high permeability of oxygen in teflon by employing a long length (~50 cm) of 1/32" teflon tube as a prevalve sample loop. The teflon loop would be inserted in a good oxygen scavenger solution (such as Cr^{+2} or V^{+2}) that could even be warmed to speed up the gas transfer equilibria. This way the sample would not need a separate purge cycle, but could be sampled directly, and degassed simultaneously. This principle could also aid in deoxygenation of the solvent before it is pumped.

As always in HPLC, filters are used to protect the system from clogging and abrasive particles. A 5-μm stainless steel filter is used at the solvent pick up point in the reservoir and a second 2-μm filter is used between the pump and sampling valve. The column is protected from the accumulation of "real world" sample impurities by means of a 6 cm precolumn. The protective column is packed with the same bonded phase material as the analytical column, although larger particle (37-40 μm), pellicular packings are used, as these are easily and efficiently hand packed.

The columns used were all high porosity silica bonded-phase columns of 25 to 30 cm length and 4.6 mm inside diameter. The particle size was generally 10 μm (for irregularly shaped particles), although a 5 μm column was also used (with spherical particles). Surface functionalities of $-CH_2CH_2-NH_2$ (μ-NH_2), $-CH_2(CH_2)_6CH_3$ (C_8), $-CH_2(CH_2)_{16}CH_3$ (C_{18}), and $-C_6H_5$(-Ø) were evaluated for organometal separations.

Choice of Waveform

Amperometry--

The simplest detector waveform used in LCEC is constant potential amperometry. This approach allows discrimination against all nonelectroactive species as well as those active ones that have a lower redox potential than that chosen for the detection. For example, consider the use of an amperometric detector used for reducible species depicted in figure 2. By controlling the constant potential applied we can discriminate between the species whose reduction waves are depicted as A, B, and C. When a potential of E_1 is applied the detector will give a response for species A only, B, and C will not be "seen" as the potential is below their minimum decomposition potential. However, at E_2 a response will be given for both A and B so

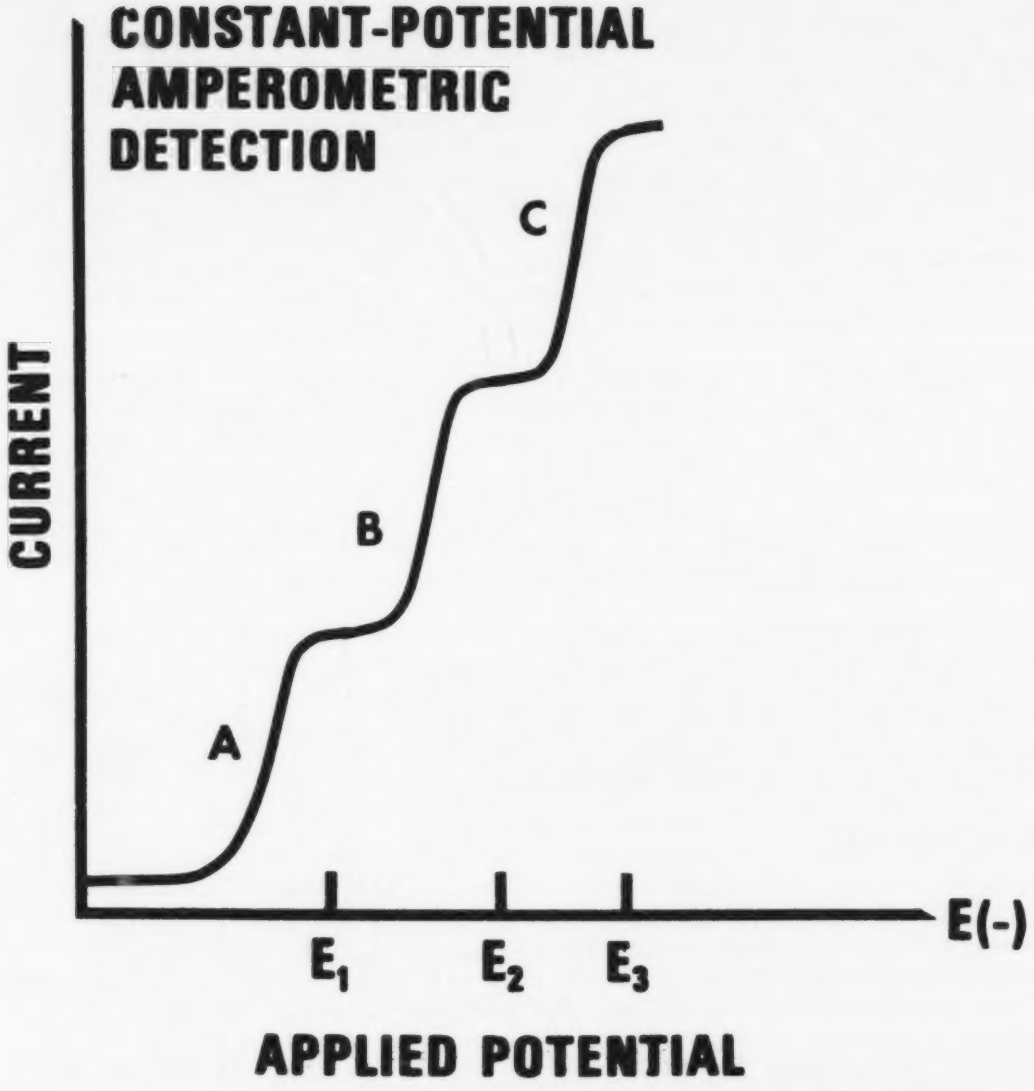


Figure 2. Amperometric detection in hydrodynamic voltammetry.

that it will be incumbent on the chromatography to separate A and B completely from each other. Component C will not be detected at either E_1 or E_2 and will not interfere. Thus, some selectivity is available in amperometric (or constant potential coulometric which is similar) detectors by selection of the potential. Some qualitative information is inherent in the amperometric LCEC measurement. The peak height will follow the amperometric current/potential function for the analyte as a function of detector potential. This can give electrochemical information about an unknown component (by repeated chromatography at different potentials) or more importantly it can confirm the presence of a known analyte in an unknown sample.

Limitation on the potential range available to the electrode in LCEC is imposed at the cathodic end by the reduction of the solvent or solvent impurities and at the anodic extreme by the oxidation of the solvent, or as in the case of the GAME, the oxidation of the electrode material. In order to examine the potential window accessible for the GAME, a point-by-point measurement of the electrolyte residual current during amperometric LCEC operation was made as a function of applied potential. Figure 3 shows this current at two pH's for a 60 percent methanol solution. At the anodic limit of about -0.2 V the oxidation of the mercury on the electrode begins. The cathodic limit is about -1.0 V for the highest sensitivity and is probably a result of the decomposition of the solvent hydrogen ions (particularly noting the current increasing effect of decreasing pH as expected for hydrogen evolution). Some of the background current, especially when small amounts of complexing agents such as 2-mercaptoethanol are used, is caused by leaching of the metals from the stainless steel. Potentials greater than about -1.2 V cannot be used in this system because the solvent faradaic background current becomes so large that even small flow pulsations and turbulence at the electrode give rise to excessive background noise, severely limiting the detection limit.

In order to evaluate the absolute detection limit for the amperometric detector, a series of dilute standard solutions were prepared. As a test organometal, trimethyllead cation was chosen because the optimum potential for its detection was near the normal cathodic potential limit of -1.0 V. In order to optimize the detection limit the peak current and background current were measured for a 3×10^{-5} M solution, as shown in figure 4. The peak currents were 32.8, 47.1, and 49.4 nA for -0.95, -0.975, and -1.00 V respectively. The highest potential (-1.0 V) gave the largest signal but the background current was much larger than at the lower potentials. Since the detector noise is proportional to the magnitude of the background current (58) the optimal potential for maximum sensitivity was -0.975 V since this would offer the best compromise between signal and noise. The dilute solutions were then analyzed at -0.975 V and also two blanks were run. The results are shown in figure 5. Examination of this data indicates that the detection limit is about 0.1 ng absolute, which translates to a concentration of 2×10^{-8} mol/L or 5 ppb, for a signal-to-noise ratio of 2. This is a fairly good detection limit as L.C. detectors go.

The linearity of the amperometric detector was demonstrated by chromatographing a series of standard solutions in the 10^{-7} to 10^{-3} mol/L range using methylmercury. The signal-to-noise ratio was optimized as before

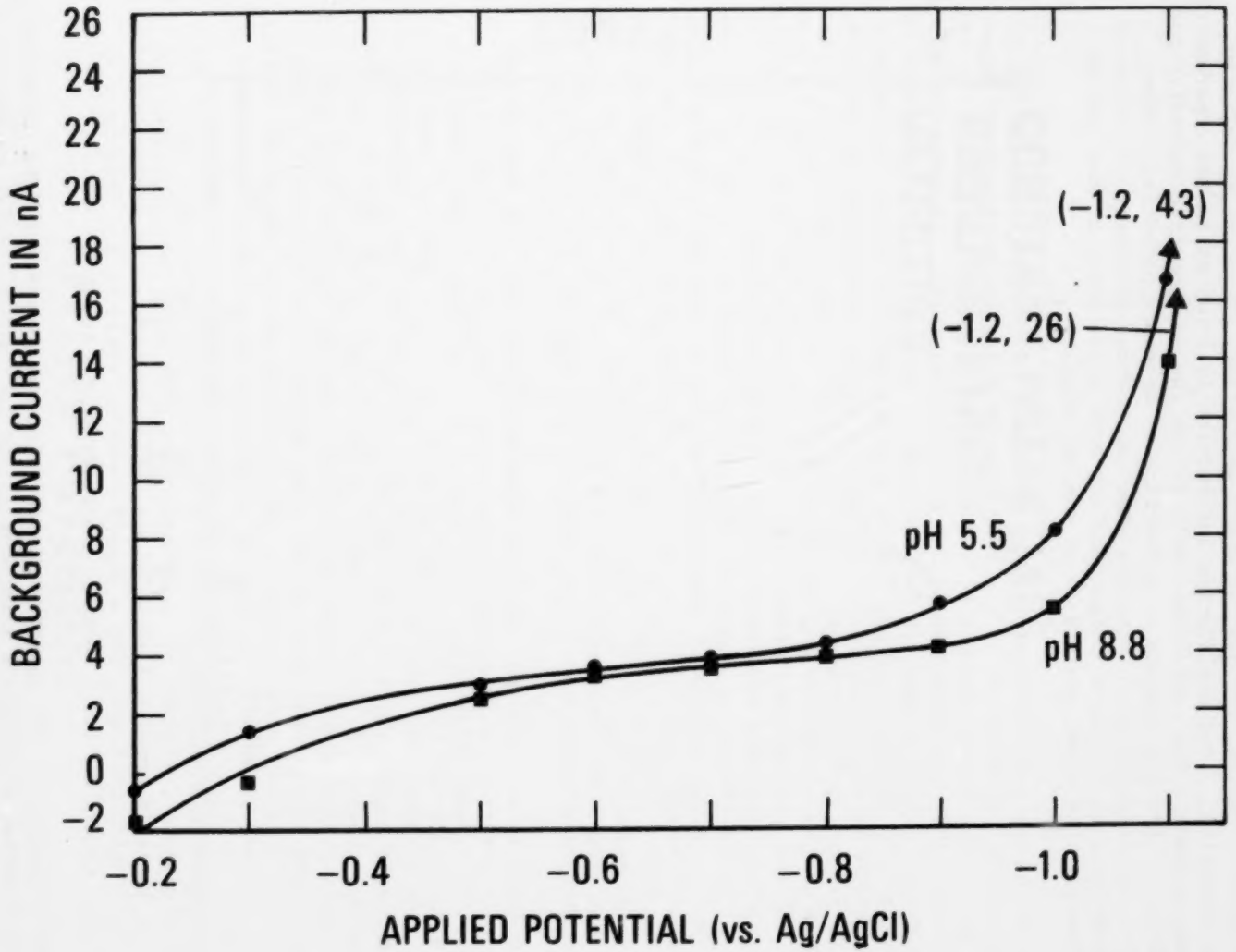


Figure 3. Background residual current as a function of applied potential. Conditions: electrode - GAME; solvent - 60% MeOH, 0.04 mol/L NH_4OAc; flow rate - 1.0 mL/min.

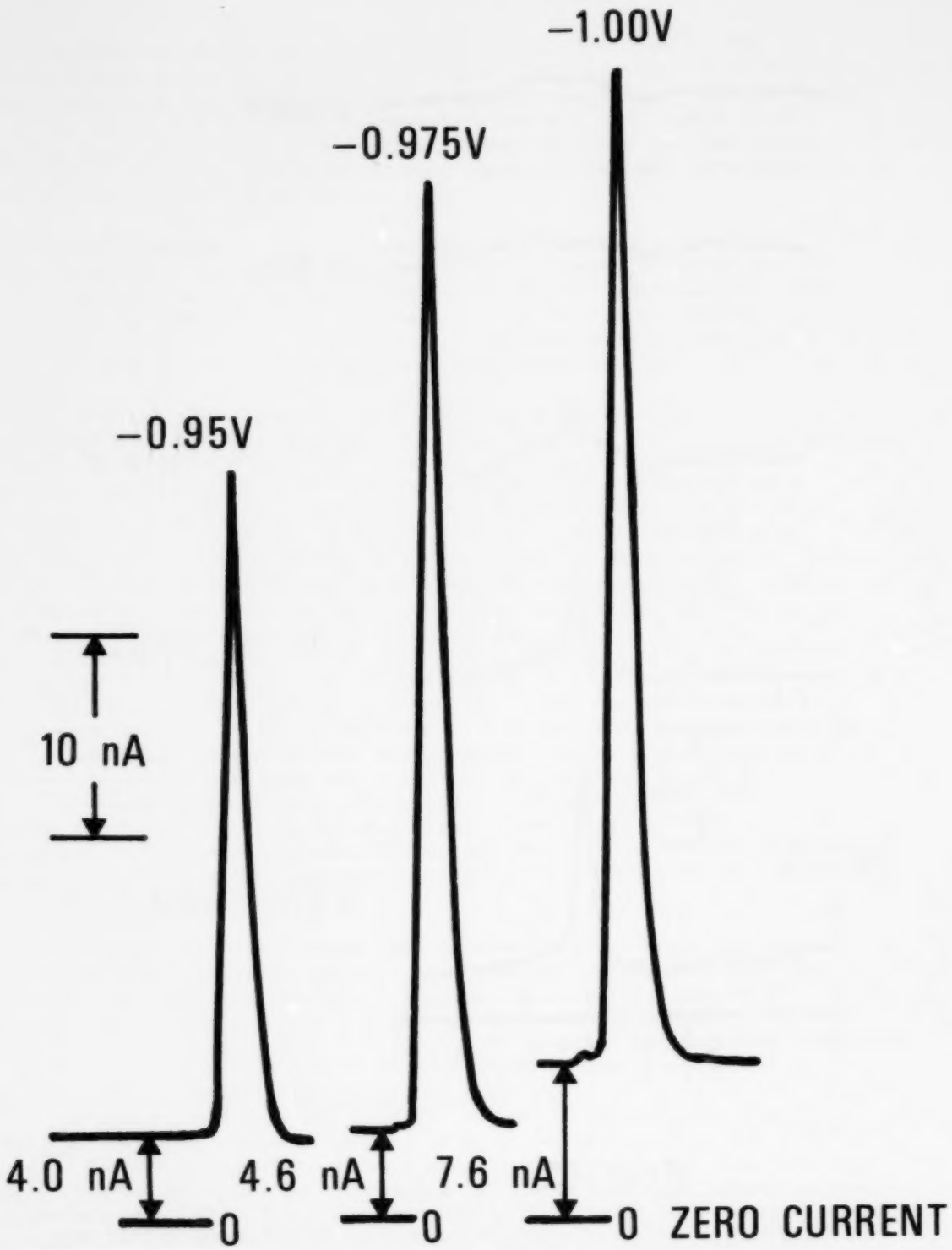


Figure 4. Optimization of detector response for $(CH_3)_3Pb^+$.

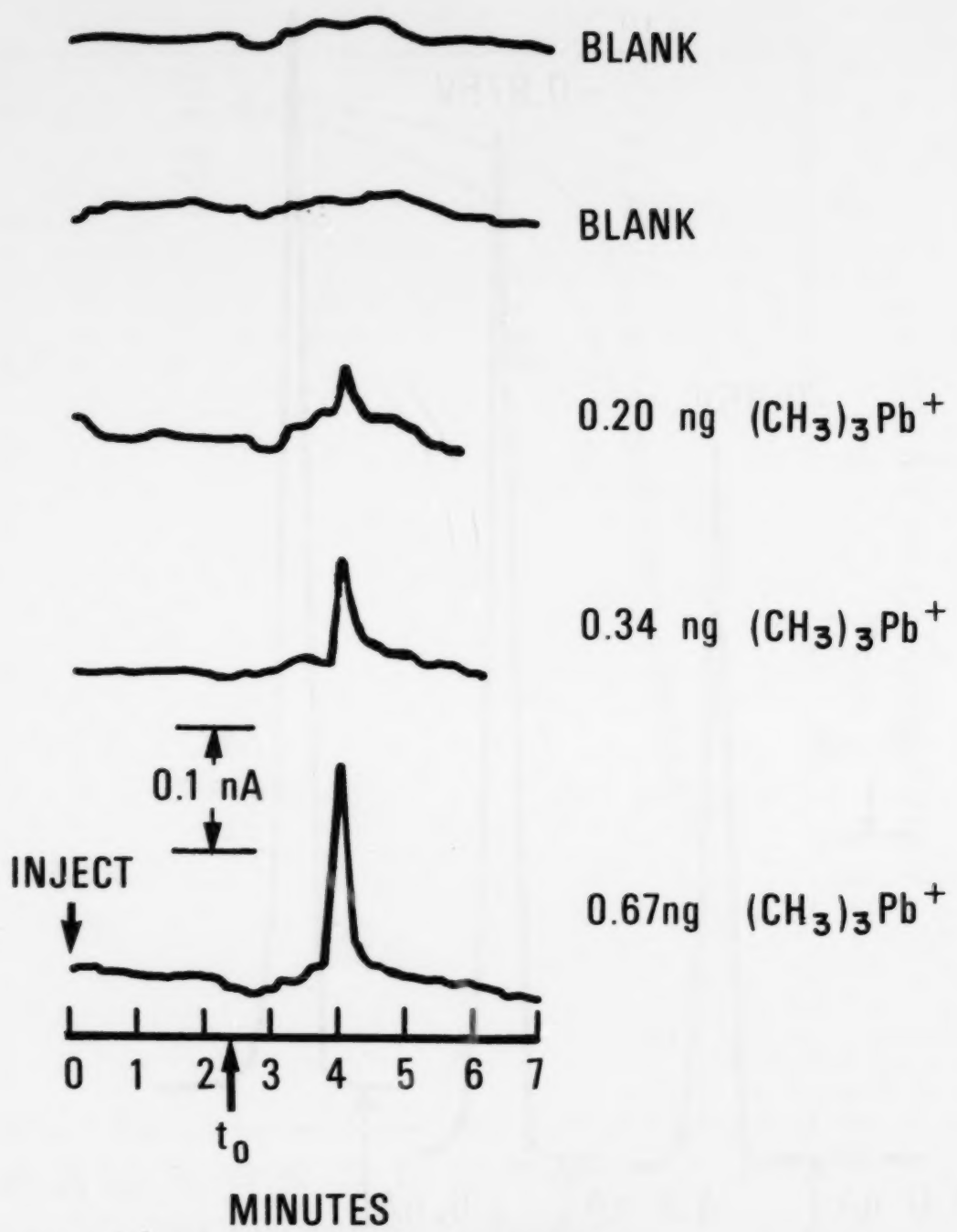


Figure 5. Limit of detection for $(CH_3)_3Pb^+$ in the amperometric mode of detection. Conditions: as figure 15 except detector potential -0.975 V.

with trimethyllead except that -0.83 V was optimal for CH_3Hg^+. The log of the peak height is plotted against the log of the concentration in figure 6. A very linear plot was obtained over 4 orders of magnitude. The data point at 1×10^{-7} mol/L was difficult to measure precisely as a result of the excessive noise present on that particular day caused by residual oxygen in the solvent. Normally, the noise is much lower so that the curve could be extended into the 10^{-8} mol/L range.

The applicability of the LCEC approach for organometals in "real" samples is going to be limited somewhat by the presence of interferences. In LCEC there are basically two systems vulnerable to interference: the chromatographic separation and the amperometric detection. To evaluate the performance of LCEC for the measurement of the organomercury species, several possible sources of interference were investigated.

The reversed phase separation of the organomercury compounds relies on the addition of a complexing agent, 2-mercaptoethanol, to the chromatographic solvent to form neutral species. Thus the presence of a stronger (or nonlabile) complexing agent in the sample would pose a chromatographic interference. To evaluate this suspicion a number of complexing agents were added to a methylmercury solution in 10 fold excess and the peak height compared to a complex-free standard. The following potential interferents, expected to be present in real samples, were evaluated: iodide and chloride (to simulate salt water samples), cyanide and sulfide (as in polluted water), cysteine (for protein sulfhydryl groups in tissue), and fulvic acid (from fresh water, decaying plant material). The fulvic acid was prepared by extracting commercially available humic acid with 10 percent methanol/water. Of the interferents tested I^-, CN^-, and Cl^- showed no interference at all. This was expected as the formation constants for the CH_3Hg^+ complex was less than that of 2-mercaptoethanol (70). However, $S^=$ forms much stronger complexes ($K_f = 10^{21.2}$) than $HOCH_2CH_2S^-$ ($K_f = 10^{16.1}$) and it was found to completely obliterate the CH_3Hg^+ chromatography. Cysteine ($K_f = 10^{15.7}$) showed only a minor (about 5 percent) decrease in peak height for a 10-fold excess. Thus, in order to have an interference-free analysis, it would be necessary to selectively remove the sulfur species from the sample.

Another type of interference that depends on the selectivity of the chromatography and the detection is the coelution of the other reducible species with the analyte species. In particular, the presence of trace metal ions in real samples could pose a problem. To assess the magnitude of the interference, a series of cations were added to methylmercury solutions. The metals that would be expected to be present in real samples and that have reduction potentials below one volt are: Hg^{++}, Cu^{++}, Cd^{++}, Pb^{++}, and Fe^{+++}. Of these metal ions Hg^{++} and Cu^{++} directly interfere by eluting at retention times very close to that of CH_3Hg^+. The presence of Cd^{++}, Pb^{++}, and Fe^{+++} would not interfere as they are chromatographically resolved from CH_3Hg^+. Figure 7-A shows an interfering mixture at the same concentration as methylmercury. This interference could be eliminated by a prior separation, improvement of chromatographic resolution, or increasing the selectivity of the detector. For example, figure 7-B shows the improvement of the chromatographic separation by a change in the chemistry of the system. A complexing

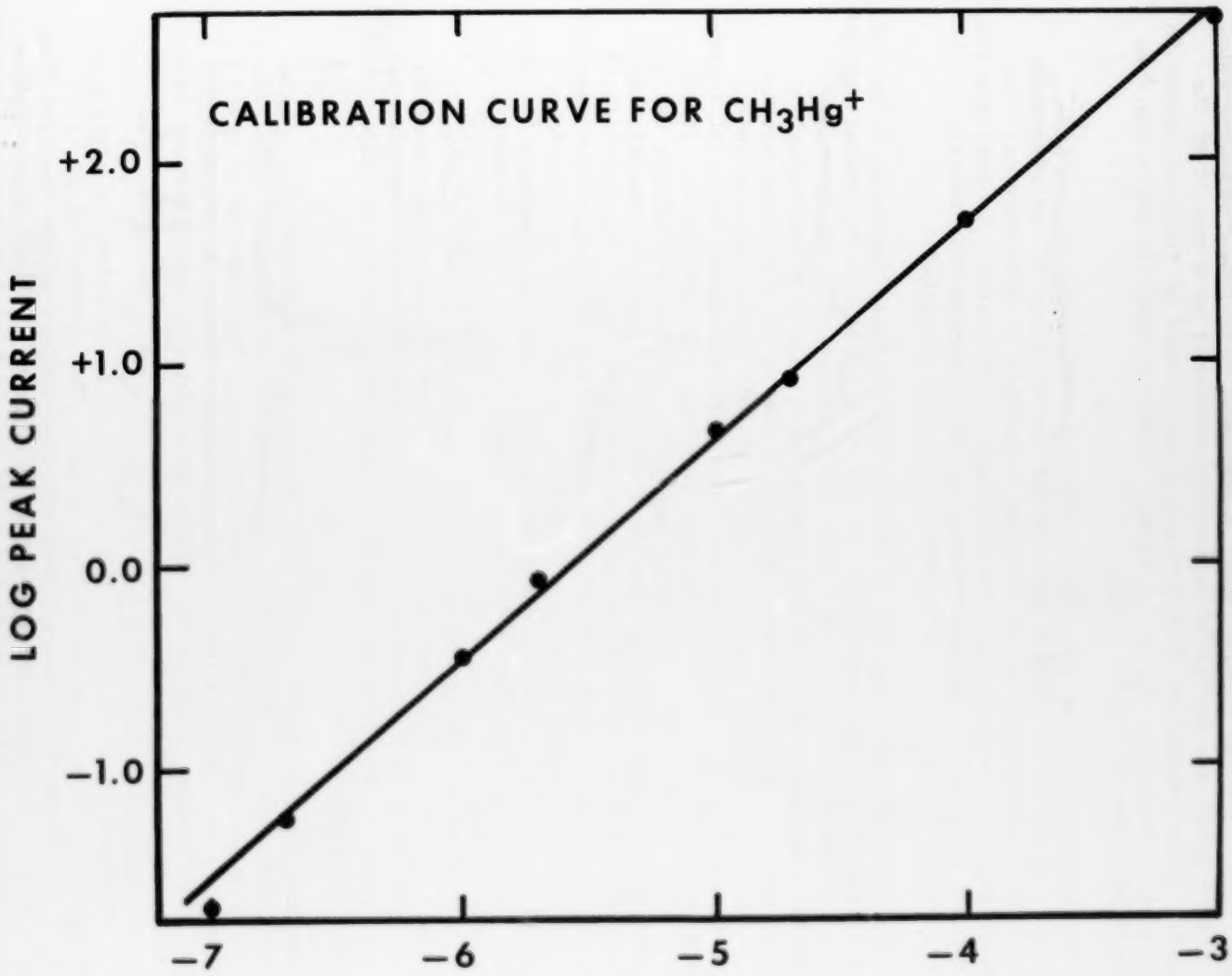


Figure 6. Calibration curve for CH_3Hg^+ in the amperometric mode of detection.

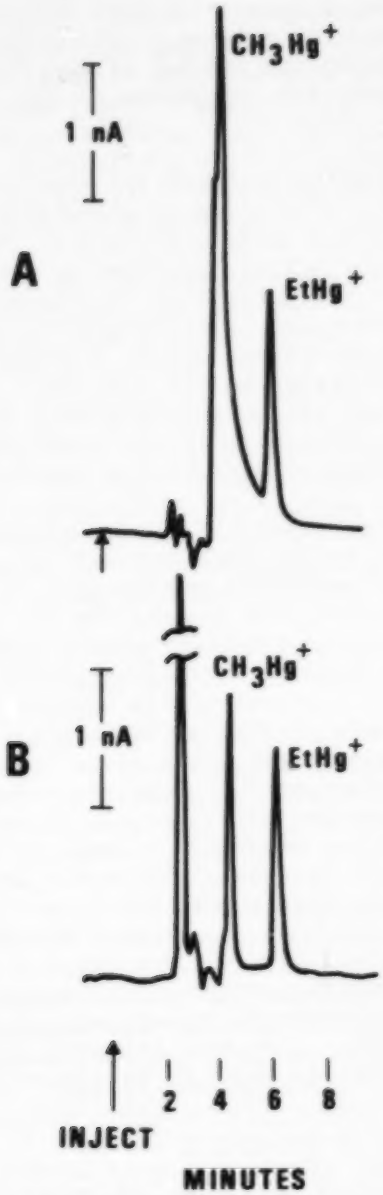


Figure 7. Elimination of the metal ion interference as organomercury measurement by improvements in the chromatography. Conditions: as figure 14 except detector potential -0.83 V (amperometric mode), sample amounts – 10^{-5} mol/L CH_3Hg^+, Pb^{++}, Cu^{++}, and Cd^{++}. Figure A without addition of EDTA; Figure B with addition of 10^{-4} mol/L EDTA to sample.

agent EDTA has been added in excess to the same solution as figure 7-A. All of the ions except the organomercury cations form stronger EDTA complexes than the 2-mercaptoethanol present in the chromatographic solvent. The anionic EDTA complexes formed apparently have little retention in the reversed phase system and elute near the solvent front, eliminating the interference. The use of a prior separation and the use of the differential pulse waveform will be discussed as alternative approaches to the metal ion interference as well.

Finally, there are possibilities of interferences directly attributable to the detection approach. Adsorption of non-electroactive species on the surface of the electrode could cause passivation. This effect was tested using benzene as a nonelectroactive, adsorbable, organic compound. An injection of a 0.1 percent V/V benzene in 50 percent methanol/water showed only a slight dip in the baseline after the solvent front, indicating the elution of benzene. This effect was caused by momentary coverage of some of the electrode surface, decreasing the available area for background faradaic reaction. However, this was such a small response for such a large amount of benzene (1000 ppm) that the potential for this particular interference in a real sample is probably not important.

As previously mentioned, the only easily variable parameter in amperometry to improve the selectivity is the applied potential. This can be used to eliminate interferences of coeluting species only if the interferents' reduction potential is greater than that of the analyte. If the interferents have lower reduction potentials they may cause a positive, additive interference in the analysis. However, it is possible to further verify that the "analyte" peak is truly a result of the analyte expected at that retention time, by repeatedly performing the analysis at different applied potentials. This works because the S shaped current-potential response function is unique, since it depends on the reversibility of the reaction, the number of electrons transferred in the electrode reaction per mole of analyte, and the decomposition potential. Thus, by measuring the peak height at several potentials for the sample and a standard solution of the analyte, the identity of the sample peak can be confirmed even for samples near the detection limit. Of course, a second detection approach can be coupled to the electrochemical detector for added qualitative information. For example, cyclic voltammetry could be used to scan the electrochemical potential range to look for other electroactive species and to confirm the presence of the analyte (61). Also such techniques as UV/visible or atomic absorption, or mass spectrometry could be used when appropriate as confirmation of analyte identity.

Differential Pulse Voltammetry--

A very promising method of improving the selectivity of the electrochemical detector is to use a differential pulse mode rather than the simple amperometry. The constant potential differential pulse detection waveform consists of 5 to 100 mV fixed pulses of 50 msec duration superimposed on a constant applied potential. The pulse repetition rate is 0.5 to 5 seconds and the current is sampled for 15 milliseconds before and at the end of each pulse. The difference of these two currents is amplified and provides the output signal.

The pulsed waveform makes the differential pulse detection much less flow sensitive (58), since the diffusion layer established only extends slightly into the solution when compared to the D.C. waveform. The lower flow sensitivity puts less constraint on the LCEC system. The control of pump pulsations is less critical and the magnitude of the background current is less crucial. The detection limit in most cases is now limited by capacitative charging current caused by the potential pulses rather than flow fluctuations (58) except when the background faradaic current is large. The background current fluctuations begin to limit the sensitivity in this system using the differential pulse detection at potentials of about -1.2 V where residual currents become large.

The most important virtue of differential pulse detection is the added selectivity of the approach. Only electroactive species that have their redox wave within the pulse height used will be sensed. Figure 8 illustrates the selective detection of an analyte species in the presence of another coeluting, more easily reduced, interferent. At the applied potential ($E_{applied}$) and the pulse height used (ΔE) only the analyte showed a marked change in its reduction current, where the interferent shows no change in current before and at the end of the pulse. Thus the differential readout will give a signal for only the species with a changing current over the pulse range, the analyte. A much greater selectivity is obtained with the differential pulse detection as it would be impossible to eliminate this interference in constant potential amperometry, even by varying the potential.

In order to experimentally evaluate the differential pulse waveform the sensitivity, linearity of response, and selectivity were examined.

The limit of detection was measured for methylmercury by analyzing a series of dilute stock solutions. Several parameters were optimized to achieve the greatest sensitivity in differential pulse operation. The signal-to-noise ratio was measured for three pulse heights and these are plotted in figure 9. The best sensitivity would be achieved with the maximum pulse height 100 mV, however, the selectivity is the least because of the large pulse range. For some applications the 10, 25, or 50 mV pulse height could be used to enhance the selectivity with only a small penalty in sensitivity. The pulse cycle time was varied from 0.5 to 5 seconds. Little difference in sensitivity was noted, but the effective time constant of the 2 and 5 second sampling rates was too long causing loss of chromatographic resolution. Since maximum resolution is important, the 0.5 second pulse repetition rate was used. Finally, the potential that gave the maximum signal was found by repeated injection of a dilute solution of CH_3Hg^+, illustrated in figure 10. Little difference in the background noise was noted over this narrow potential range, so the maximum signal became the only criterion for potential selection. Figure 11 shows some solutions very near the detection limit obtained under the optimum conditions. This indicates a detection limit of about 1×10^{-8} mol/L which is 2.5 ppb (aqueous) or 0.04 ng (for a 20 µL sample) for methylmercury. The sensitivity of the differential pulse waveform is thus very similar to that of amperometric detection in our system.

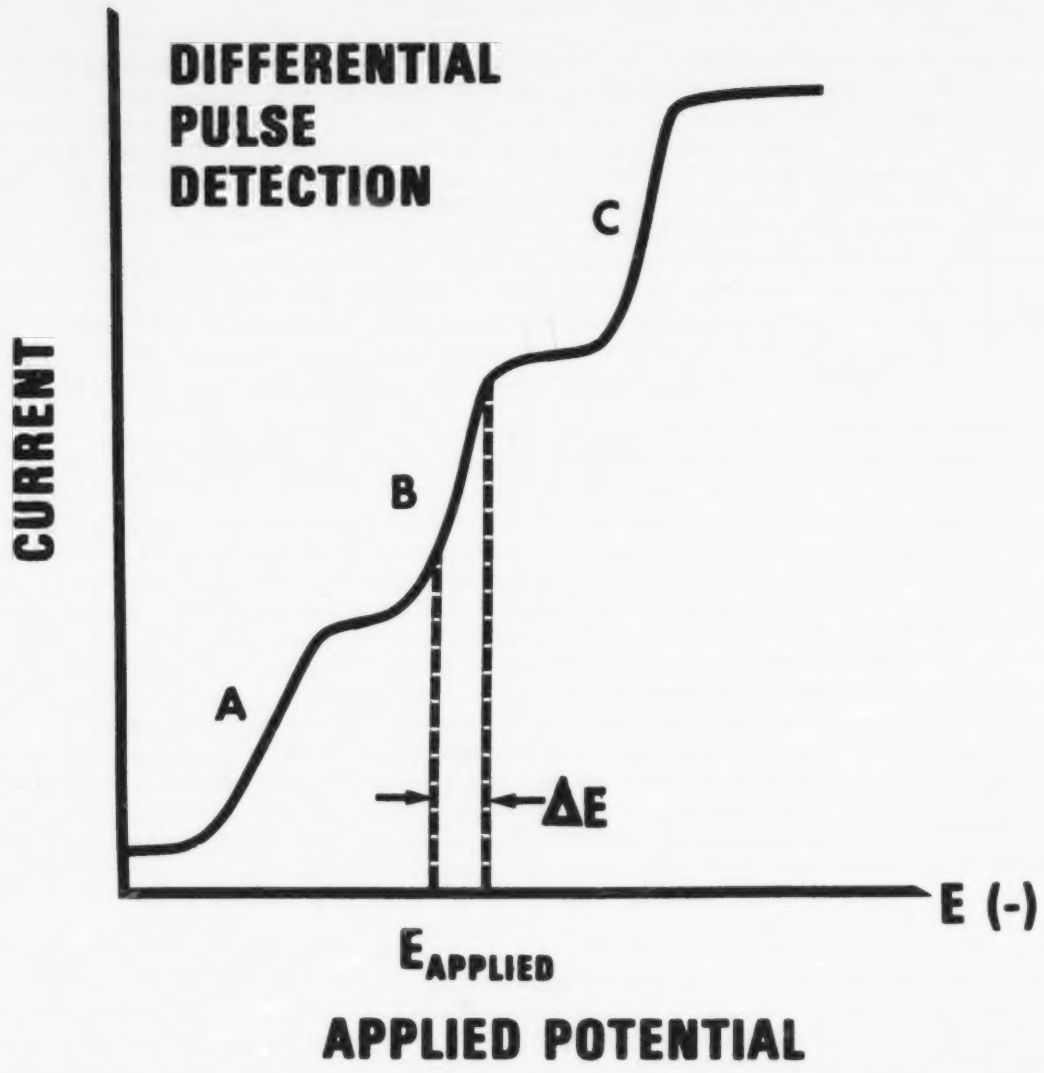


Figure 8. Differential pulse detection in hydrodynamic voltammetry.

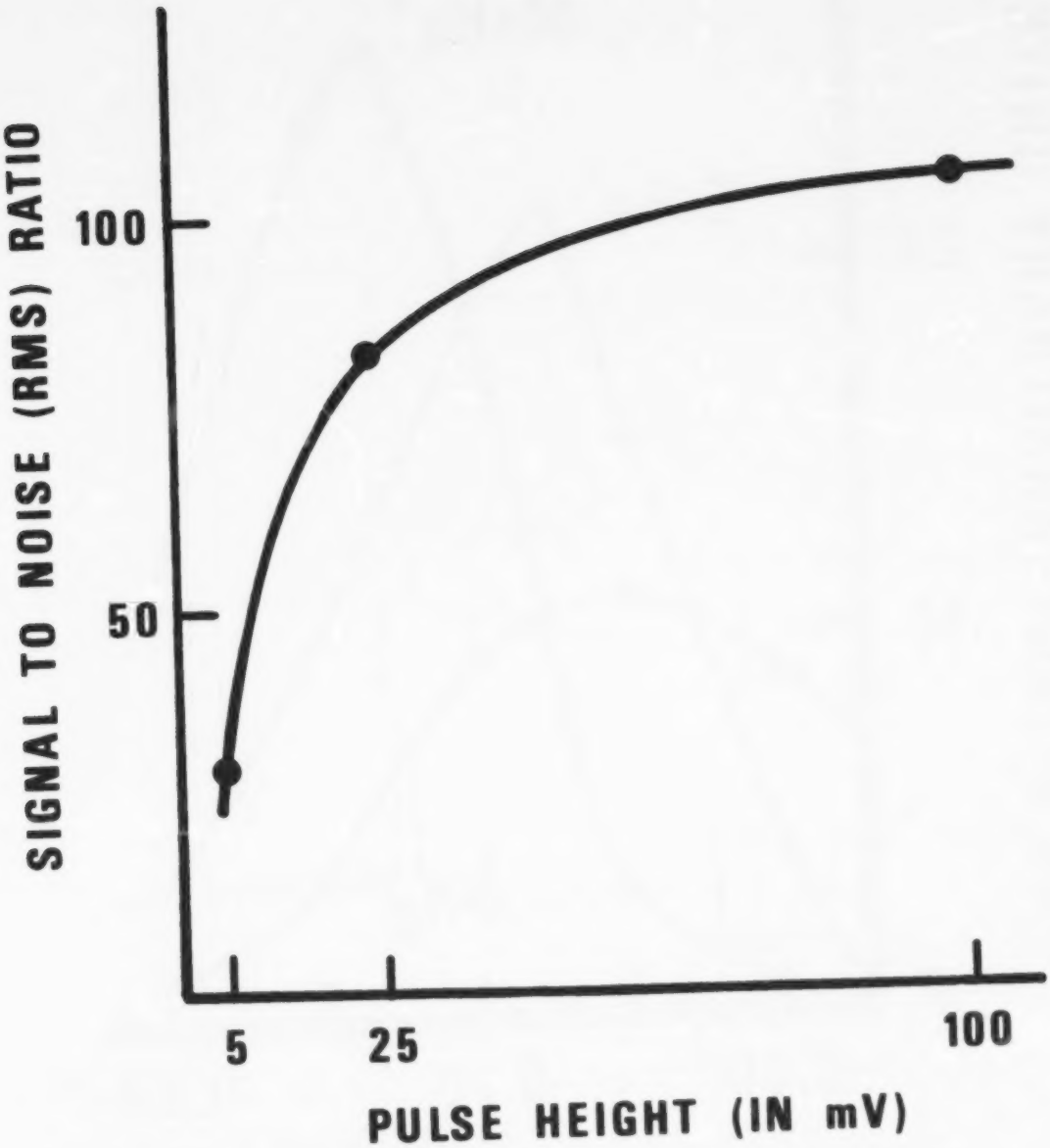


Figure 9. Relationship between signal-to-noise ratio and pulse height in differential pulse detection. Conditions: analyte - 5×10^{-7} mol/L CH_3Hg^+, pulse time - 0.5 sec, time constant - 0.0 sec.

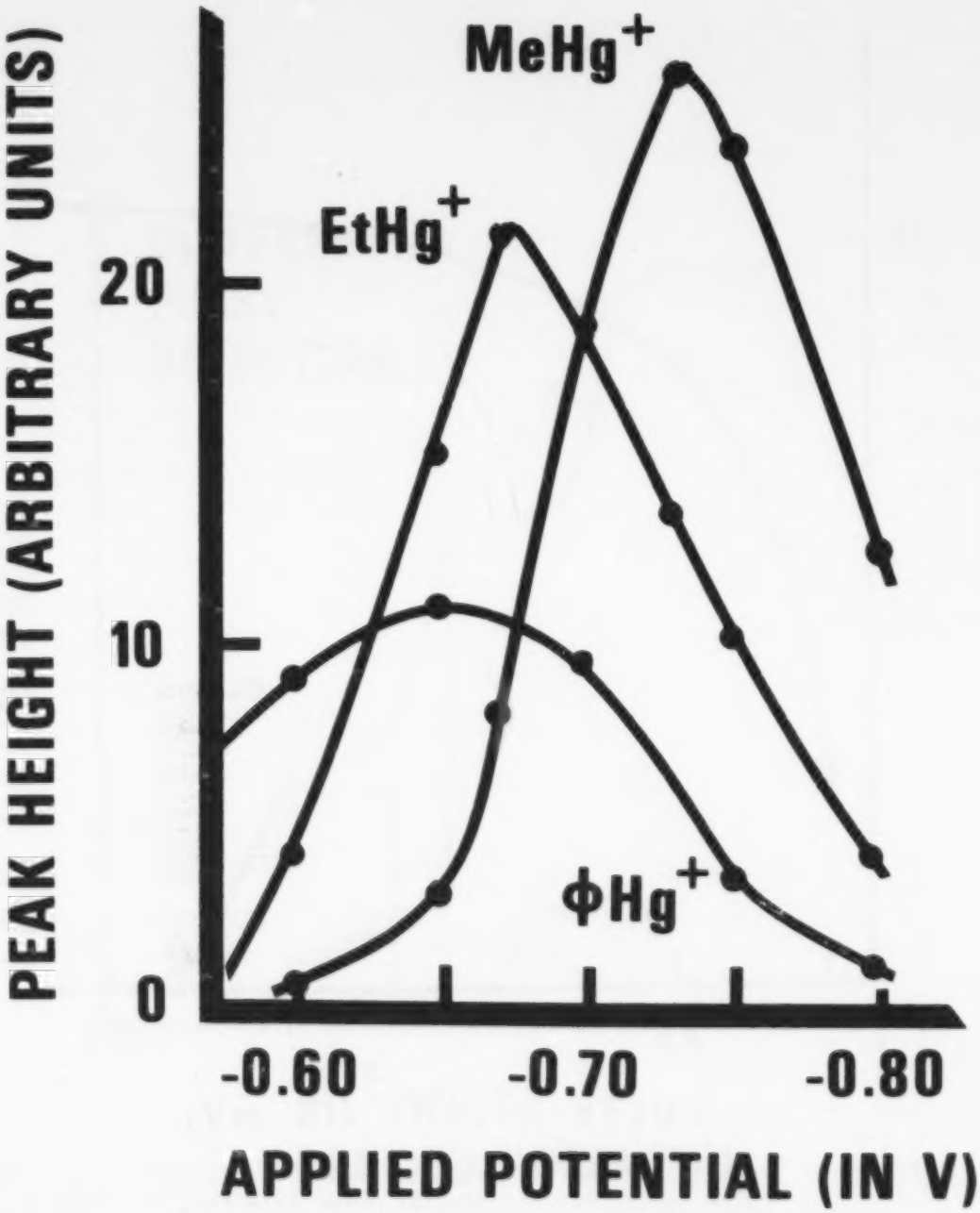


Figure 10. Differential pulse current response as a function of applied potential. Conditions: pulse height - 5 mV, pulse time - 0.5 sec, time constant - 0.0 sec.

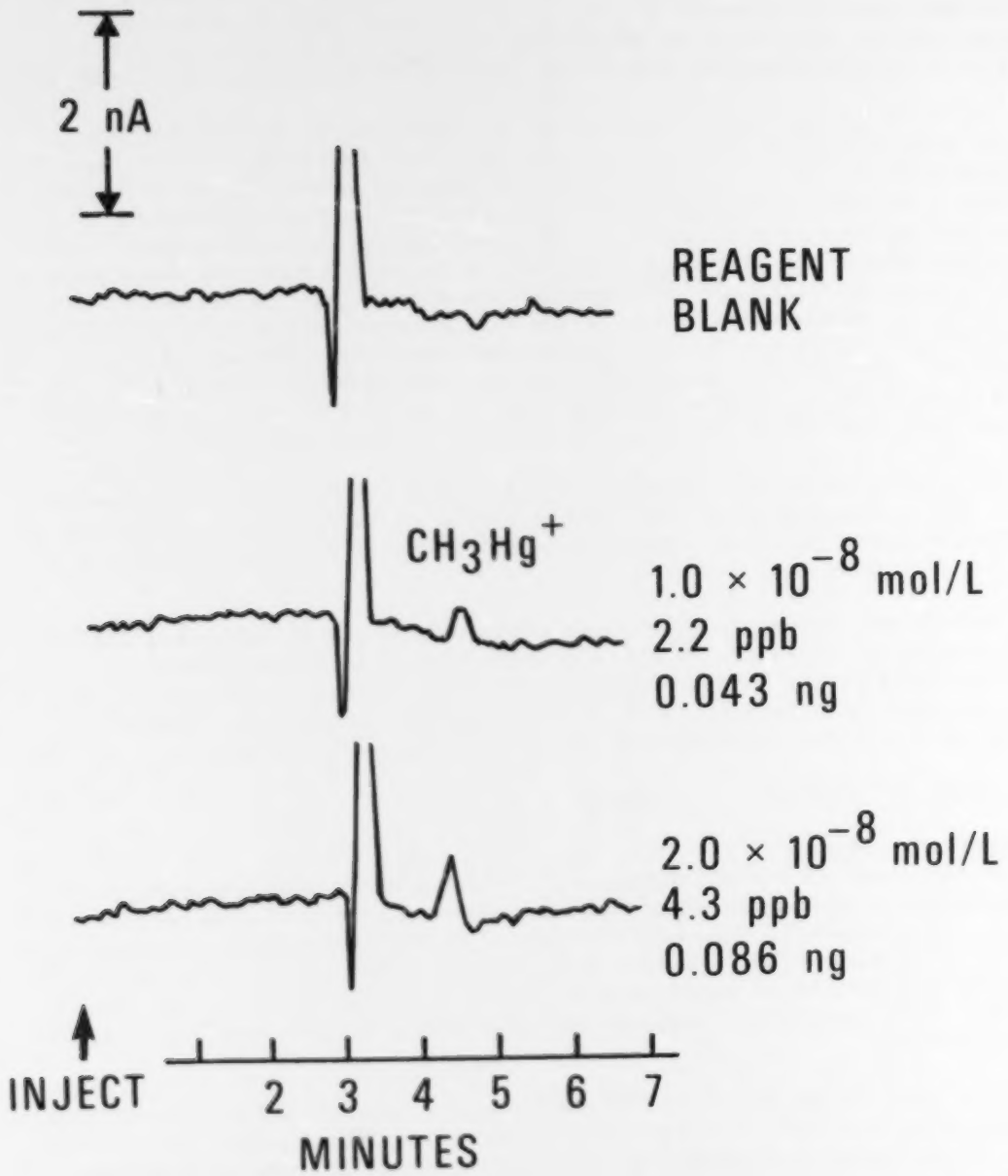


Figure 11. Limit of detection for CH_3Hg^+ in the differential pulse mode of detection. Conditions: detector potential - -0.75 V, pulse height - 100 mV (-), time constant - 0.3 sec.

The selectivity of the differential pulse measurement approach was tested by the addition of several interfering ions to a methylmercury solution. Figure 12 shows a comparison of the results for a mixture of Cu^{++}, Cd^{++}, Pb^{++}, $CH_3CH_2Hg^+$, and CH_3Hg^+ all at the 5×10^{-6} M level, first in the amperometric, then in the differential pulse mode of operation. Serious interference could be seen using the simple D.C. mode but the differential pulse waveform shows high selectivity for CH_3Hg^+ and $CH_3CH_2Hg^+$.

The selectivity of differential pulse detection is so high for an analyte that I like to call it "species specific" detection. Using this approach samples can be analyzed with a minimum of sample "cleanup" before analysis. However, this approach limits the number of simultaneously detectable species to only three or four, unless the detector potential is programmed during the chromatographic run to be reoptimized for each analyte. The potential switching approach may be feasible as the electrode settling time (sort of a capacitative and faradaic time constant) is quite rapid in this mode of operation. In the case of very similarly behaved (electrochemically) species such as methyl-, ethyl-, and phenylmercury, it is possible to choose a compromise potential (-0.70 V) that will allow simultaneous detection (as previously illustrated in figure 10).

The last test was the linearity of response in the differential pulse mode. Stock solutions of CH_3Hg^+ were prepared by volumetric dilution and a calibration curve was run. The results in figure 13 show good linearity over 3 orders of magnitude.

The two waveforms are both quite comparable in their detection limits and linearity of response. They offer the analyst a choice between "species specific" selectivity for the differential pulse approach and simultaneous multicomponent "promiscuity" with the amperometric detection. Thus the optimum waveform will be dependent on the sample to be analyzed.

Development of Separation Conditions

Organomercury Cations--

An important aspect of any liquid chromatographic analysis scheme is the development of optimum conditions for the separation of the analyte species. There must be adequate resolution of all the analyte components from each other and from potential interferents. The correct separation mechanism must be chosen based on the chemistry of the analytes and interferents. Variation in the mobile phase's solvent strength can "fine tune" the separation.

When electrochemical detection is used, the type of separation mechanism is limited to partition (reverse-phase) and ion exchange. This is a result of the requirement of a conducting, very polar solution, needed for the detection system. The solvent must contain a supporting electrolyte, using at least a 0.05 M solution of an ionic salt as a conductor, to lower the solution resistance and prevent migration currents. The commonly used solvents that are suitable for electrochemical detection are water, methanol, acetonitrile, and dimethyl-sulfoxide. As supporting electrolytes salts, buffers, or complexing ions may be used, dependent on the chromatography.

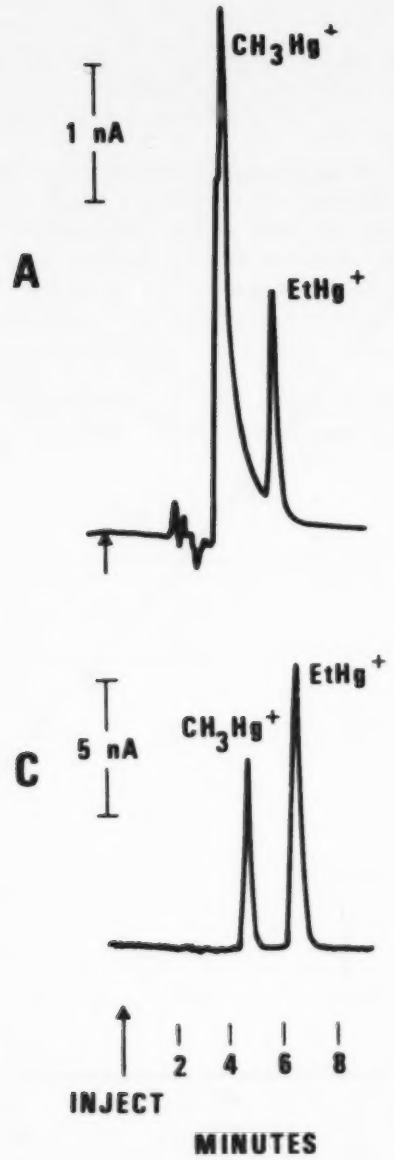


Figure 12. Elimination of metal ion interference by increasing the selectivity of the detector. Conditions: Figure A as Figure 7A, Figure B as Figure A except detector mode - differential pulse, potential - -0.70 V, pulse height - 25 mV (-), pulse time - 0.5 sec, time constant - 0.3 sec.

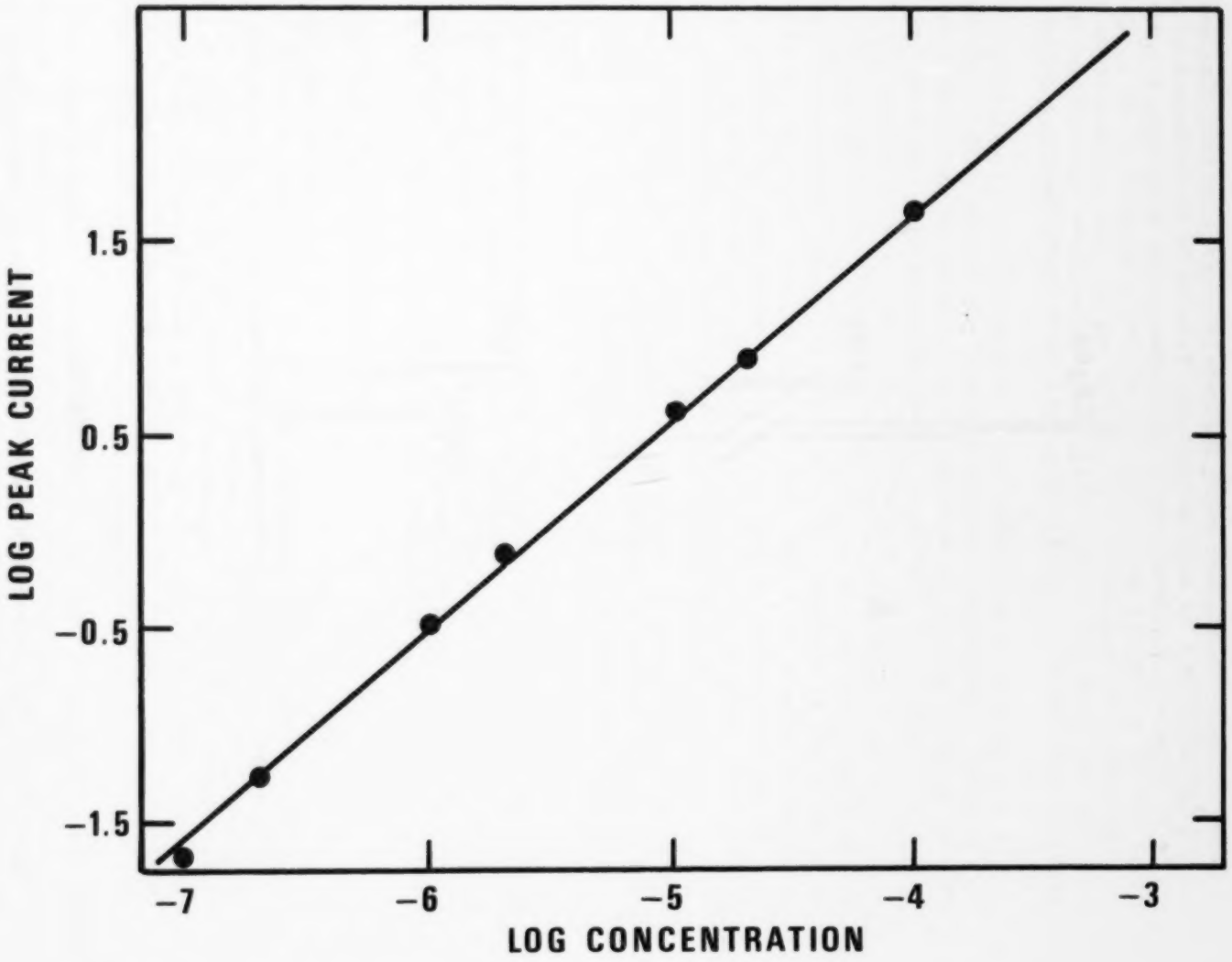


Figure 13. Calibration curve for CH_3Hg^+ in differential pulse mode of detection.

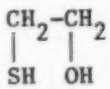
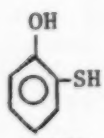
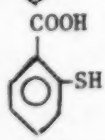
Although the solvent choice in LCEC is somewhat limited, this provides very little handicap in the analysis of organometals, as partition and ion exchange are the logical separation mechanisms for these moderately polar and ionic analyte species.

The organomercury species (CH_3Hg^+, $CH_3CH_2Hg^+$, $C_6H_5Hg^+$ and even Hg^{++}) were chosen as the first group for separation. Initially, methylmercury was used as a test compound for various chromatographic approaches.

Direct injection of CH_3Hg^+ in reverse-phase systems using a μNH_2 and C_8 bonded phase columns yielded poor results. It was reasoned that CH_3Hg^+ was too ionic in the acetate medium (K_f for a neutral acetate complex is only $10^{3.6}$) to be efficiently separated in the reverse-phase system. In order to favor neutral CH_3Hg^+ species, a strong complexing agent was needed. The strongest methylmercury complexes are formed by mercaptans and sulfide (71, 72, 70).

A comparison of several polar mercaptans as complexing ligands was made using a reverse-phase C_{18} column. Table 7 summarizes the results.

TABLE 7

Reagent added 1% V/V	Structure	k'	N(plates/M)	Characteristics of peak
2-mercaptoethanol		1.63	7600	sharp, some tailing
2-mercaptophenol		--	--	retained infinitely
2-mercaptobenzoic acid		1.85	4400	sharp, tailing
4-mercaptoaniline		2.96	850	broad, excessive tailing

(Continued)

TABLE 7 (Continued)

Reagent added 1% V/V	Structure	k'	N(plates/M)	Characteristics of peak
1-mercaptoethylether	CH_2CH_2O | | SH CH_2CH_3	3.33	160	broading, excessive tailing
1-mercaptoheptane	$CH_2(CH_2)_5CH_3$ | SH	4.51	1200	broad with tailing
3-mercaptopropanoic acid	CH_2CH_2-COOH | SH	1.26	2960	sharp but excessive tailing
1-mercaptoglycerol	CH_2-CH-CH_2 | | | SH OH OH	1.76	2160	sharp with some tailing

Based on this data 2-mercaptoethanol was chosen as the complexing ligand. The separation was further optimized by the selection of a high performance C_{18} column material and adjustment of the percentage of organic solvent (73).

The optimum conditions for organomercury analysis were: column — Spherosorb ODS (C_{18}) (5 μm), solvent — 40 percent methanol/water with 0.06 mol/L NH_4OAc, pH 5.5, flow rate 1.0 mL/minute and 0.01 percent V/V 2-mercaptoethanol. The separation is illustrated in figure 14.

Multielement Separation--

One particularly promising column material for the separation of organometals is the bonded-phase micro-NH_2. Figure 15 shows a good separation of several different organometals in a 40 percent methanol/water solvent. This separation illustrates quite nicely the multicomponent capabilities of the amperometric mode of detection.

Organoleads--

Some good results were obtained with the organolead species on the Spherosorb C_{18} column with 80 percent methanol/water with a trace of mercaptoethanol as shown in figure 16. Surprisingly the addition of strong complexing agents to the samples had no effect on the retention of the organoleads shown. The following ligands were tested: EDTA, diethyldithiocarbamate, dithizone, halides, and cyanide. This would seem to indicate that the complexing agents do not compete with the mercaptoethanol ligand. The

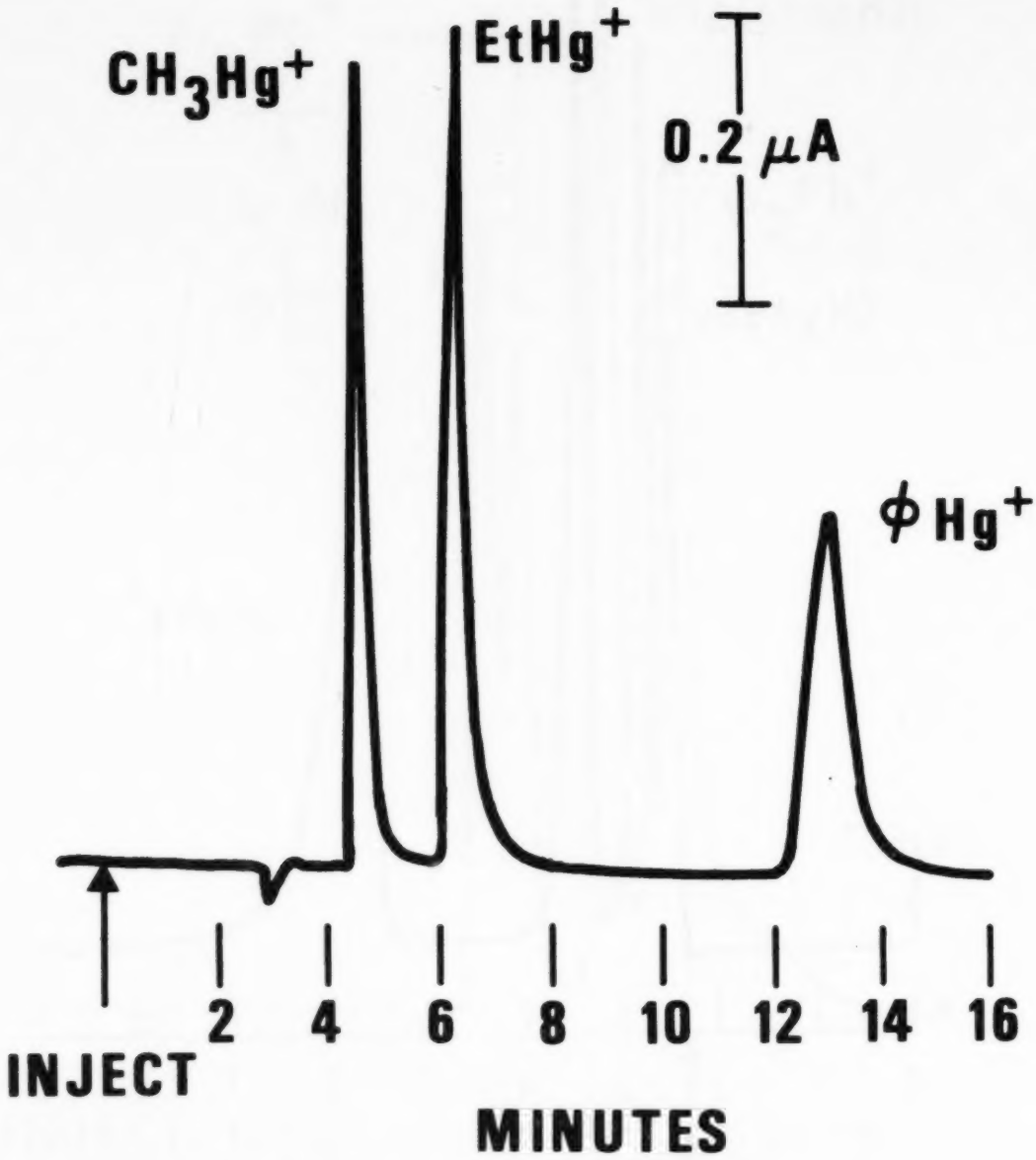


Figure 14. Separation of organomercury cations by charge-neutralization reverse-phase chromatography. Conditions: column — Altex Spherosorb ODS 5 μm, 4.6 x 250 mm; solvent — 40% MeOH, 0.06 mol/L NH_4OAc pH 5.5, 0.01% ME, flow rate — 1.0 mL/min; detection mode — differential pulse; potential — -0.70 V; pulse height — 25 mV (-); pulse time — 0.5 sec.

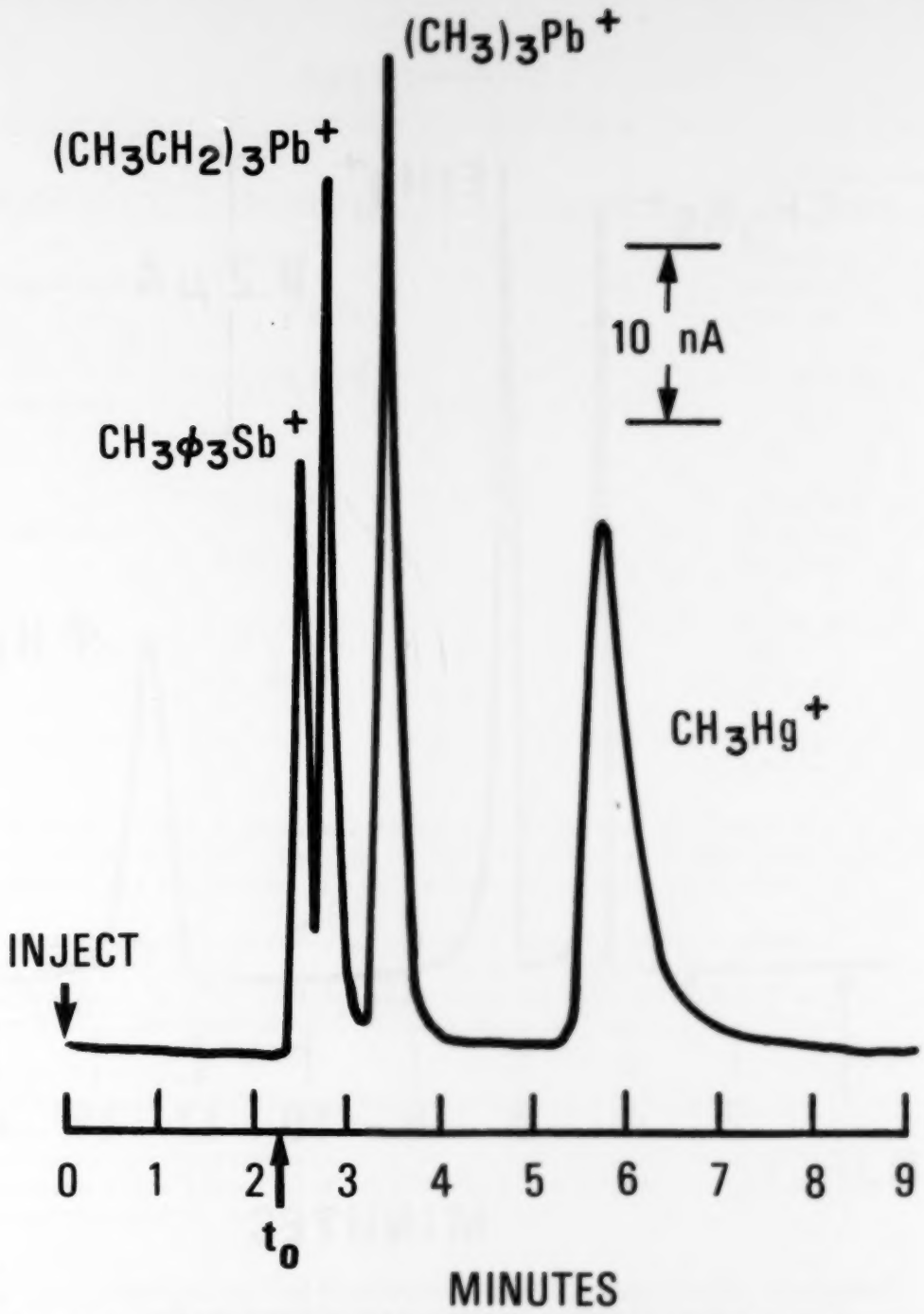


Figure 15. Separation of selected organometals. Conditions: column — Altex μNH_2 10 µm, 4.6 x 250 mm; solvent — 40% MeOH 0.06 mol/L NH_4OAc pH 5.5; flow rate — 1.0 mL/min; detector mode — amperometry; potential — -1.00 V, time constant — 1.0 sec.

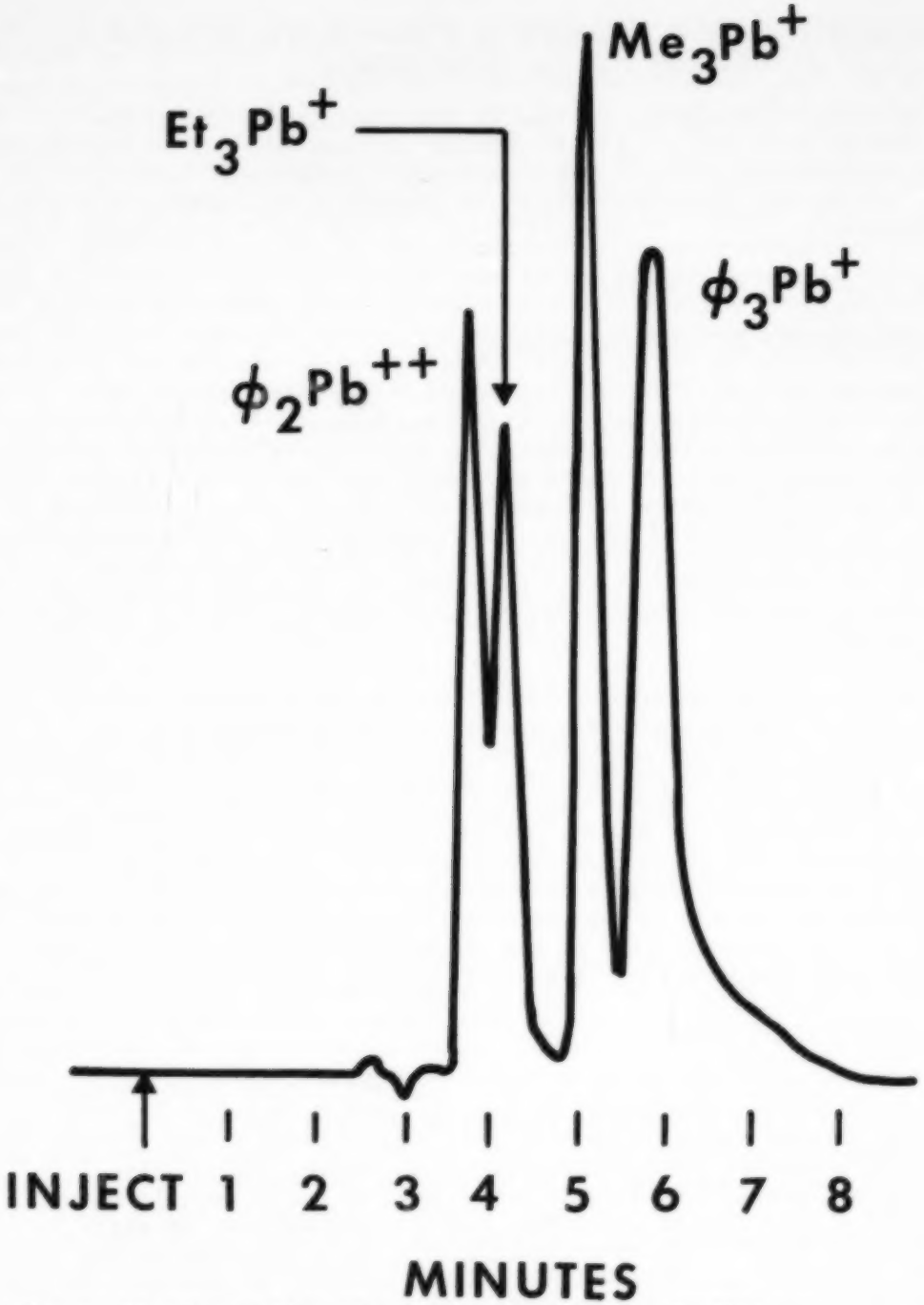


Figure 16. Separation of organolead cations. Conditions: column – Altex Spherosorb ODS 5 µm, 4.6 x 250 mm; solvent – 80% MeOH, 0.04 mol/L NH_4OAc pH 5.5 0.001% ME; flow rate – 1.0 mL/min, detection mode – amperometry; detector potential – -0.95 V; sensitivity 50 na/V; sample concentration – 5 x 10^{-5} mol/L of each.

complex-insensitivity of this separation is a good feature, as ligands in the sample would not interfere with the chromatography.

Work on chromatographic separations of trace metal ions and of the organotin species is underway. Little success has been achieved in the separation of the organotins in reversed phase systems. Perhaps more careful examination of the complexation chemistry of organotins will point the way to their separation.

APPLICATION OF LCEC TO REAL SAMPLES

Special Considerations in Sampling Organometals

Quantitative removal of the analyte from a "real world" sample is always an important problem in any analytical measurement. Organometal ions have some characteristics that make them particularly difficult to extract. The compounds are not stable to strong acid (due to hydrolysis) or oxidizing agents (73), which are commonly used in wet ashing procedures for trace metals (74) in solid samples. For organometals, only homogenization with an extractant solution or alkaline hydrolysis of the matrix can be used (5). It is not advisable to heat homogenization mixtures strongly as organometallic species for form neutral, volatile complexes and can be lost in the gas phase.

Organometal species can also be lost from prepared homogenate (or sample) solutions. Some organometals are subject to photolysis (3), so that it is important to store the solutions as prepared in the dark. These analytes may also be lost on container walls when in very dilute solution. With glass containers, unacidified solutions can lose trace metal ions by exchange for surface hydrogen ions on the Si-O-H groups of the glass. Perhaps cationic organometal ions could follow the same path. Organometals are readily lost from aqueous solutions by adsorption on plastic containers (75,76) such as polyethylene because of the hydrophobilicity of the organic portion of the analytes. A thorough study of the storage of organometals is needed and will provide future work in this project.

Natural Water Preconcentration

In order to monitor the levels of toxic organometals in water samples, very sensitive detection methodology is needed. Even in heavily polluted waterways the filtered sample is usually quite low in heavy metals (total) relative to the suspended particulate material and underlying sediment (77,7). Although levels of some toxic metals such as mercury may be in the low parts-per-billion range in the water, living organisms such as algae, plankton, and ultimately fish can accumulate this small amount up to the parts per million range in their tissues. Thus, it would be important to measure even very low levels of organometals in water samples.

In order to make analytical measurements in the low and sub parts-per-billion (ng/g) range, it is necessary to preconcentrate the organometals

before analysis. There are four major approaches to metal ion preconcentration that could be considered for organometals. Direct concentration by low pressure evaporation can be used but losses on the container walls or by volatilization, combined with the increase in the concentration of interferences, suggest this is not a good approach in this case. Coprecipitation can be used sometimes, where a metal ion is carried down with an added precipitation reagent. This often gives irreproducible results since it is too dependant on nucleation conditions. Also it gives a sample with a large excess of a potentially interfering precipitant. Solvent extraction is a very good method, where neutral complexes of the ion are extracted into an immiscible organic phase. This is particularly attractive for organometals because of their organophilicity. For example, methylmercury may be preconcentrated as its neutral chloride complex from a dilute HCl solution into toluene. However, it is difficult to use solvent extraction for preconcentrations greater than a factor of 10 unless the distribution coefficient of the analyte is quite large ($>10^3$) and the solubility of the organic solvent is quite low. Also it is quite difficult to completely equilibrate two phases at volume ratios much greater than 10 to 1. For higher preconcentration ratios (100 to 1000) a column procedure is often very effective. For organic compounds or neutral metal ions, a nonpolar stationary phase can be used (78). Ionic species may be retained on ion exchange materials. The analyte may be analysed directly on the column (as with neutron activation analysis or x-ray fluorescence) or by elution in a very small volume of solvent with the proper stripping reagent. Thus, column preconcentration is the approach we have chosen for the organomercury species.

The choice of column packing material is very important as it must quantitatively retain the organomercury species even though large volumes of sample elute through. Cation exchange would not be a good choice for the monocationic organomercury ions as these have a low charge-to-size ratio. However, neutral halide complexes can be made and retained on a nonpolar stationary phase in an approach chemically similar to the solvent extraction approach.

The basic apparatus for the column preconcentration of methyl- and ethylmercury is shown diagrammatically in figure 17. A 100 mL water sample is acidified with 0.10 mL of concentrated nitric acid (making it 1.6×10^{-2} M) to help prevent adsorption by formation of neutral hydroxy or other basic complexes. The sample is collected in an acid washed borosilicate glass bottle with a Teflon liner in the cap. The sample is filtered through a 8 µm Millipore Teflon filter and any solid material in the filter is washed with several small portions of 1.6×10^{-2} M HNO_3 and the washings combined with the sample. One milliliter of 2 M ammonium citrate pH 3.1 is added to the sample bringing the final buffered pH to 2.5. The water sample is then placed in the sample reservoir. The second reservoir, shown in the figure, holds a solution of 1 M halide (I^-, and Cl^- have been used). The feed rate of sample to halide solution is 10 to 1 going into the pump. This ratio is controlled by the inside diameter of the tubing used to connect the two reservoirs to the "Tee" joint. A single piston metering pump (LDC minipump) provides a flow rate of 2.8 ml/minute to the column. A short (6 mm long, 3.2 mm inside diameter) column is packed with either Rohm and Haas XAD-2 (120-170 mesh) or with Waters Associates Styragel 500A.

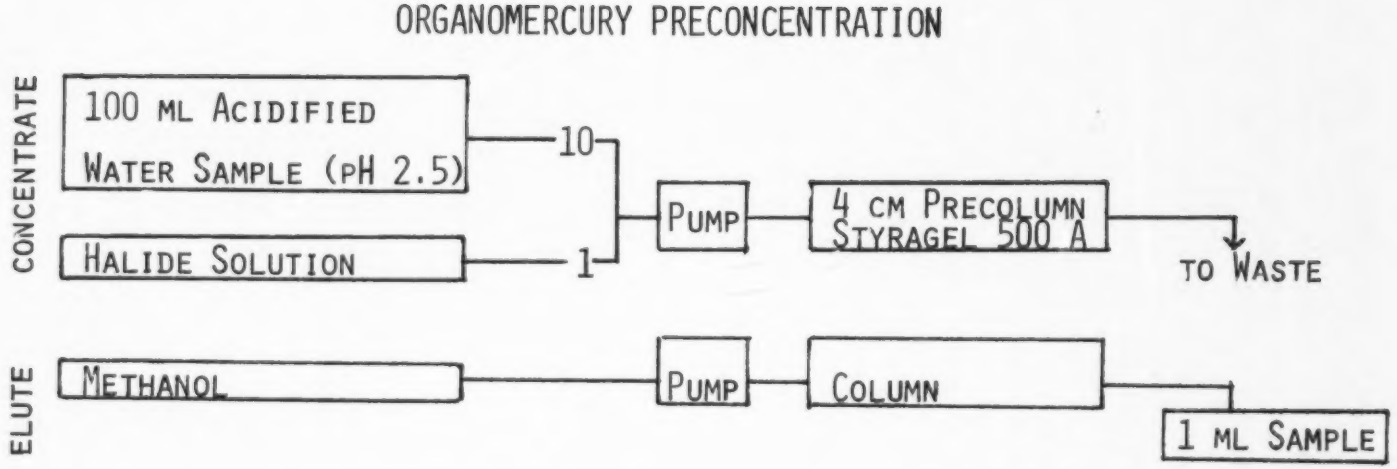


Figure 17. Block diagram of organomercury preconcentration.

Both materials are polystyrene providing a nonpolar surface for adsorption of the neutral organomercury halide complexes. The sample is pumped through the column and then the solvent is switched to methanol at a flow rate of about 0.1 mL/minute. This elutes the neutral complexes in a very small volume of 1.00 mL (the first 200 µL corresponding to V_m is discarded first). If the recovery was 100 percent this set up would give a preconcentration factor of 100. However, recoveries were not quantitative. When I^- is used the recoveries are: CH_3Hg^+ 95 percent, $CH_3CH_2Hg^+$ 70 percent (n=5).

Although the recovery to date is not quantitative, it is sufficiently reproducible (about ± 5 percent) to be useful for water samples where the precision of the result is not critical. To evaluate the utility of the approach and to study the potential interferences, several synthetic sample mixtures were prepared. Previous investigation showed that sulfide ion and certain heavy metal ions would interfere with the amperometric LCEC analysis. However, the conditions of the preconcentration are such that these interferences should be eliminated. Metal ions should not be retained in the concentration step unless they form strong neutral complexes in dilute, acidic, halide solution. Although many metals do form halide complexes, the anionic complexes are not protonated to neutrality except in high concentrations of acid. For example, the quantitative extraction of Fe^{+3} into ethyl ether requires 6 M HCl (79):

$$Fe^{+3} + 4Cl^- + H^+ \rightarrow HFeCl_4$$

To test the metal ion interference a solution of 10^{-5} M Cu^{++}, Cd^{++}, Hg^{++}, Fe^{+3}, and Pb^{++} and 10^{-6} M CH_3Hg^+ was preconcentrated using the iodide procedure above. No concentration of the interfering metal ions occurred and the expected recovery of the organomercury species was obtained.

The complexing agent interference was studied by addition of a 10 fold excess (10^{-5} M) of sulfide and cyanide to the organomercury species. These anions should not interfere at pH 2.5 as they will be converted to their neutral acids (H_2S and HCN) and be unable to complex the CH_3Hg^+ (70). No interference was experimentally observed.

At this point a real sample was attempted, so a portion of NBS pond water was collected, acidified, filtered, and analysed. No organomercury species were found to the 100 ppt (parts per trillion) level. An identical sample was spiked with the analytes, and the expected recovery was obtained for CH_3Hg^+ and $CH_3CH_2Hg^+$. A particularly complex sample, genuine seawater was also analysed. A 100 mL sample of Danish Standard Seawater P_{49} (chlorinity 19,370 °/₀₀) was spiked and analysed. The spiked amount was successfully recovered, but no organomercury cations were found in the seawater.

Certainly, this preconcentration approach shows promise, but further work is needed to improve recovery and verify the reproducibility. Also it should be possible to extend the procedure to include $C_6H_5Hg^+$ and $(CH_3)_2Hg$ with little modification. The general approach should be useful for other organometals as well.

Methylmercury in Fish

There is concern for the levels of mercury (and other heavy metals) in aquatic organisms, especially those which are frequently part of the human diet. Most aquatic species are good bioaccumlators of metals, and although the metal ion level may be in the ppb range in the surrounding water, the organisms may have tissue levels in the ppm range. The Environmental Protection Agency toxic "action level" for mercury in fish is 0.5 ppm.

In order to provide a standard material to aid analytical researchers in comparing techniques for trace analysis in fish, the NBS has prepared a research material of lyophilized albacore tuna fillet. The material has been carefully homogenized and packaged in a nitrogen atmosphere to prevent decomposition. Analysis results for total mercury indicates 0.95 ± 0.1 ppm in the freeze dried sample (80). Our task was to evaluate the amount of methylmercury in the material. We also made measurements of the methylmercury levels in a sample of lyophilized shark paste prepared by the Department of Agriculture of the University of Tokyo (81).

Removal of organometals from solid material such as tissue poses several problems. It is not possible to use oxidative, acidic digestion to remove the organic material as the methylmercury will decompose. Present methodology uses either alkaline digestion or aqueous homogenization to provide a workable liquid sample (5).

We have investigated a new sampling approach for methylmercury in tissue. This organometal ion will be completely protein-bound in tissue by cysteinyl groups (70). The sampling approach was based on competitive complexation with 2-mercaptoethanol. The ME forms slightly stronger complexes ($K_f = 10^{16.1}$) than cysteine ($K_f = 10^{15.7}$) (9), and therefore could compete for the CH_3Hg^+ in the tissue.

For the analysis, a fresh 2.00 g sample of the lyophilized tuna was weighed into a 50 ml pyrex centrifuge tube and about 7 g of 50 percent methanol containing 0.1 percent mercaptoethanol was added. Two samples were prepared as above and four more were spiked with known amounts of methylmercury. The samples were homogenized with a Brinkmann Polytron sonic/mechanical homogenizer for about 5 minutes. Then they were warmed for 45 minutes to 60°C (boiling chloroform) to hasten the equilibrium. The tubes were then centrifuged at high speed for 10 minutes and the supernatant liquid (∿3 mL) was taken for LCEC analysis.

The measurement of the extract was made in the "species specific" differential pulse mode of detection, optimized for CH_3Hg^+. The selectivity for methylmercury is quite good. The identity of CH_3Hg^+ in the tuna was further substantiated by analysis at an alternate potential where the expected signal would be about 2/3 of maximum. The sample showed the same potential dependence as a standard.

For the quantitative analysis the standard additions curve for the differential pulse mode was drawn. A reasonably linear result was obtained with $r = 0.9977$. From the graph methylmercury was found to be approximately

0.97 ppm in the sample. This result was very close to the 0.95 ± 0.1 ppm value for total mercury (80) and would seem to indicate that all of the mercury present is methylmercury. No ethylmercury could be detected, and inorganic Hg^{++} would not be detected under these conditions. However, the uncertainty in the CH_3Hg^+ value is fairly large by this sampling method as the absolute recovery of CH_3Hg^+ is only 0.35 ppm by direct comparison to standards. This low recovery (about 35 percent) could reflect the equilibrium competition between sample sulfhydryl groups and the mercaptoethanol or the distribution of the neutral CH_3HgME complex between the solution phase and the oily solid phase. If this is the source of the low recovery, the interference would be multiplicative (at least for standard additions close in concentration to the analyte). Thus the recovery should be 35 percent for both sample analyte and standard added CH_3Hg^+ and at least an approximate result can be obtained by this approach. A much more reliable result could be obtained if the recovery could be improved to greater than 80 percent by the development of a stronger mercapto-complexing agent for CH_3Hg^+.

Another approach we briefly investigated was selective oxidation of the methylmercury-complexing, sulfhydryl groups of the tissue protein. If these groups could be oxidized (mercaptans are quite easily oxidized to sulfates) the methylmercury would be set free. Unfortunately, I have yet to find a reagent that will oxidize sulfhydryl groups that won't oxidize CH_3Hg^+. Dilute H_2O_2 and I_2 both oxidize cysteine and methylmercury. Perhaps weaker agents such as Sn^{+4} and $Fe(CN)_6^{-3}$ will do the job selectively and rapidly.

The standard procedures presently used for the measurement of methylmercury in tissue involve alkaline hydrolysis to remove most of the solid matrix. The hydrolysis is followed by acidification with hydrochloric acid. The methylmercury cation can then be extracted from the aqueous solution with toluene as the neutral chloride complex. The procedure employed in this work for the measurement of methylmercury follows the recommendation of the Analytical Methods Committee of the Chemical Society (5), with some modification. The sample and reagent amounts were reduced by 4/5. The aqueous back-extraction solution used was 0.01 mol/L disodium thiosulfate (82) buffered to pH 5.5 with 0.05 mol/L ammonium acetate. This extraction solution was compatable with our chromatographic separation, and analysis was performed directly on this aqueous extract, eliminating the final toluene extraction.

In all cases a standard additions procedure was used, with known amounts of diluted CH_3Hg^+ solution added to the solid material before the hydrolysis step. Duplicates of each sample and of two standard additions were run. Figure 18 shows duplicate injections of two portions of the shark sample. The relative average deviation is 0.75 percent.

Table 8 shows the result of the methylmercury determination in the two fish samples. The values obtained are in fairly good agreement with the values obtained by other methods for total mercury. The high proportion of methylmercury to total mercury is consistent with the results of other workers (84,85,86).

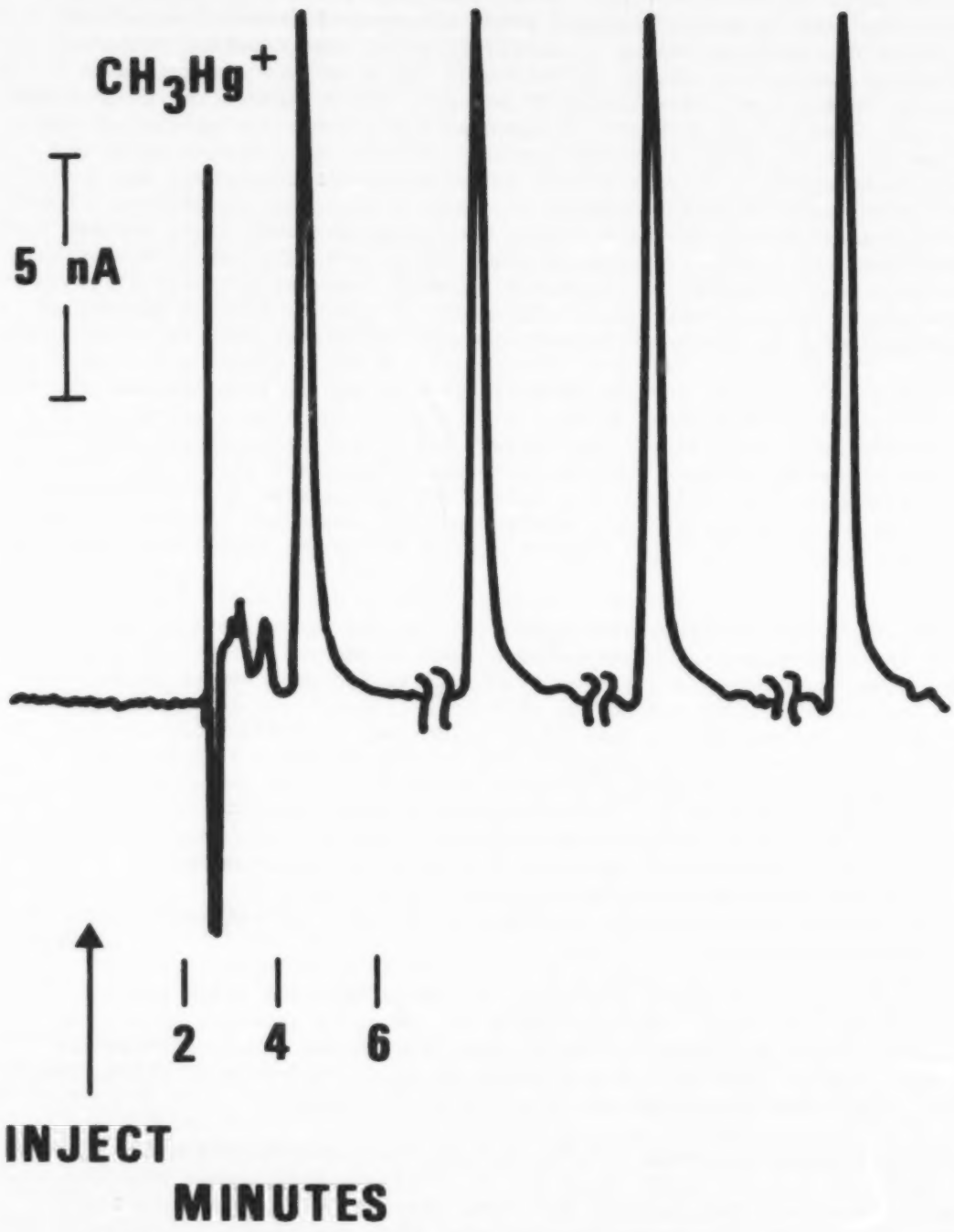


Figure 18. Determination of Methylmercury in Shark Paste.

TABLE 8

Sample	CH_3Hg^+ in ppm	Total Hg
Research material 50	0.93 ± 0.1	0.95 ± 0.1
Shark paste	8.4 ± 0.1	7.4 (83)

The accuracy of these results are totally dependent on the purity of the commercial CH_3HgOAc (Alfa Inorganics), stated as 99 percent. However, reliable standards are needed to improve the certainty of the values reported in Table 8. One approach to the preparation of a methylmercury standard solution could be controlled-potential coulometry. Any Hg^{++} impurities can be prereduced at low applied potential, then the potential can be stepped to that necessary for the first methylmercury reduction. The total current flow is measured, and through coulombs law the number of moles of methylmercury could be calculated. Future work will examine this approach to the preparation of a standard.

Conclusion

The electrochemical detection approach to liquid chromatography promises to provide a selective and sensitive method for the determination of organometal cations in environmentally important samples with a mimimum of sample preparation.

REFERENCES

1. Thayer, J. S. J. Organometal. Chem., 76:265, 1974.

2. Tobias, R. S. Organometal. Chem. Rev., 1:93, 1966.

3. Jewett, K. L., F. E. Brinkman, and J. M. Bellama. Marine Chemistry in the Coastel Environment. ACS Symp. Series #21, 1975. p. 305.

4. Baughman, G. L., J. A. Gordon, N. L. Wolfe, and R. G. Zepp. Chemistry of Organomercurials in Aquatic Systems. EPA publication #660/3-73-012, 1973. p. 32.

5. Analytical Methods Committee, Analyst, 102:769, 1977.

6. Ridley, W. P., L. J. Dizikes, and J. M. Wood. Science, 197:329, 1977.

7. Brinckman, F. E., and W. P. Iverson. Marine Chemistry in the Coastal Environment. ACS Symp. Series #18, 1975. p. 319.

8. Brinckman, F. E., G. E. Parris, W. R. Blair, K. L. Jewett, W. P. Iverson, and J. M. Bellama, Environmental Health Perspectives, 19:11, 1977.

9. Handbook of Chemistry and Physics, 51st Edition, CRC Press, 1971.

10. Zimak, J., and E. Cwiertniewski. Roczn. panst. Zakl. Hig., 27:19, 1976.

11. Cappon, C. J., and J. C. Smith. Anal. Chem., 49:365, 1976.

12. Ohkoshi, S., T. Takahoshi, and S. Toshio. Japan Analyst, 22:593, 1975.

13. Knof, H., and G. Albers. Erdoel Kohle Erdas Petrochem. Brennst.-Chem., 28:534, 1975.

14. Robinson, J. W., E. L. Kiesel, J. P. Goodbread, R. Bliss, and R. Marshall. Anal. Chim. Acta, 92:321, 1977.

15. Parris, G. E., W. R. Blair, and F. E. Brinckman. Anal. Chem., 49:378, 1977.

16. Talmi, J. Anal. Chim. Acta, 74:107, 1975.

17. Reamer, D. C., T. C. O'Haver, and W. H. Zoller. Methods and Standards for Environmental Measurement. NBS Special Publication #464, 1977. p. 609.

18. Vastogh, A. Z. Anal. Chem., 279:366, 1977.

19. Graffeo, A. P., C. J. Riggie, and B. L. Sgontv. Proceedings of 4th Annual Symposium on Environmental Monitoring, paper 202.

20. Floyd, M., and S. E. Sommers. Anal. Lett., 8(8):525, 1975.

21. Hancock, S., and A. Slater. Analyst, 100(1191):422, 1975.

22. Stary, J., and J. Prosilova. J. Radiochem. Radioanal. Lett., 26(1):33, 1976.

23. Van Loon, J. C. Chemical Speciation Studies Using Atomic Absorption and Fluorescence Detectors with Chromatography and Other Separational Techniques. NBS Analytical Symposium, Feb. 14, 1978.

24. Botre, C., F. Cocace, and R. Cozzani. Anal. Lett., 9(9):825, 1976.

25. Epstein, M. private communication, 1978.

26. Brinckman, F. E., W. R. Blair, K. L. Jewett, and W. P. Iverson. J. Chrom. Sci., 15:493, 1977.

27. Mairanovskii, S. G. Russian Chem. Rev., 45(4):298, 1976.

28. MacCrehan, W. A., R. A. Durst, and J. M. Bellama. Anal. Lett., 10(14): 1175, 1977.

29. Kemula, W. Roczn. Chem., 26:281, 1952.

30. Robertus, R. L., R. J. Cappell, and G. W. Bond. Anal. Chem., 30:1825, 1958.

31. Van Duyne. R. P., and D. A. Aikens. Anal. Chem., 40:254, 1968.

32. Buchanen, E. B., and J. R. Bacon. Anal. Chem., 39:615, 1967.

33. Blaedel, W. J., and J. W. Todd. Anal. Chem., 30:1821, 1958; also, 33:205, 1961.

34. Corfield, M. C., and A. Robson. Biochem. J., 84:146, 1962.

35. Koen, J. G., J. K. Huber, H. Poppe. and G. den Boef. J. Chrom. Sci., 8:192, 1970.

36. Tustanowski, S. J. Chromatog., 31:266, 1967.

37. Stillman, R., and T. S. Ma. Microchim. Acta, 1973:491, 1973; also, 1974:641, 1974.

38. Mohnke, M., R. Schmunk, and H. Schultze. Z. Anal. Chem., 219:137, 1966.

39. Sandi, E., Z. Anal. Chem., 167:241, 1959.

40. Mann, C. K. Anal. Chem., 45:1864, 1957.

41. Kemula, W., and K. Butkiewicz. Roczn. Chem., 39:73, 1965.

42. Kemula, W., D. Sybilska, and A. Kwiecinska. Roczn. Chem., 39:1101, 1965.

43. Kemula, W., B. Behr, K. Chlebicka, and D. Sybilska. Roczn. Chem., 39:1315, 1965.

44. Kemula, W., D. Sybilska, and K. Chlebicka. Roczn. Chem., 39:1499, 1965.

45. Kemula, W., and D. Sybilska. Anal. Chim. Acta, 38:97, 1967.

46. Kemula, W., and J. Witwicki. Roczn. Chem., 29:1153, 1955.

47. Kemula, W., and Z. Stachurski. Roczn. Chem., 30:1285, 1956.

48. Brook, A. J. Chem. Ind., 48:1434, 1968.

49. Gierst, L., and W. Dubru. Bull. Soc. Chim. Belges, 63:379, 1954.

50. Joynes, P. L., and R. J. Maggs. J. Chrom. Sci., 8:427, 1970.

51. Wasa, T., and S. Musha. Bull. Chem. Soc. Japan, 48(7):2176. 1975.

52. Fleet, B., and C. J. Little. J. Chrom. Sci., 12:747, 1974.

53. Kissinger, P. T., C. Refshange, R. Dreiling, and R. N. Adams. Anal. Lett., 6:465, 1973.

54. Johnson, D. C., and J. Larochelle. Talanta, 20:959, 1973.

55. Johnson, D. C., and R. J. Davenport. Anal. Chem., 46:1971, 1974.

56. Taylor, L. R., and D. C. Johnson. Anal. Chem., 46:262, 1974.

57. Feher, Z., G. Nagy, K. Toth, and E. Pungor. Analyst, 99:699, 1974.

58. Swartzfager, D. G., Anal. Chem., 48:2189, 1976.

59. Lankelma, J., and H. Poppe. J. Chromatog., 125:375, 1976.

60. Buchta, R. C., and L. J. Papa. J. Chrom. Sci., 14:213. 1976.

61. MacDonald, A., and P. D. Duke. J. Chromatog., 83:333, 1973.

62. Durst, R. A., F. E. Brinckman, K. L. Jewett. and J. E. Doody. Methods and Standards for Environmental Measurement. NBS SP 464, 1977. p. 473.

63. Robenstein, D. L., and R. Doetre. Anal. Chem., 49:1036, 1977.

64. Heaton, R. C., and H. A. Laitenin. Anal. Chem., 46:547, 1974.

65. Adams, R. N. Electrochemistry at Solid Electrodes. Marcel Dekker Inc., 1969.

66. Vydra, F., K. Stulik, and E. Julakova. Electrochemical Stripping Analysis. John Wiley and Sons Inc., 1976. p. 141.

67. LaQue, F. L., and H. R. Copsur. Corrosion Resistance of Metals and Alloys. ACS Monograph Series #158, Chapter 15.

68. Sawyer, D. T., and J. L. Roberts. Experimental Electrochemistry for Chemists. John Wiley and Sons, Table 7-4, 1974.

69. Freeman, D. H. private communication (1977).

70. Zepp, R. C., G. L. Baughman, N. L. Wolfe, and D. M. Cline. Environ. Let., 6:117, 1974.

71. Simpson, R. B. J. Am. Chem. Soc., 83:4711, 1961.

72. Schwarzenback, G., and M. Schellerberg. Helvetica Chim. Acta, 48:28, 1965.

73. MacCrehan, W. A. Ph.D. Thesis, University of Maryland, College Park, MD, 1978.

74. Maienthal, E. J., and D. C. Becker. A Survey of Current Literature on Sampling, Sample Handling, and Long Term Storage for Environmental Materials. NBS TN 927, 1976.

75. Youssef, M. K., A. Sina, A. A. Kassem, S. A. Ilrahin, and M. A. Attia. Indiam J. Pharm., 35:155, 1973.

76. Brinckman, F. E. private communication 1977.

77. deGroot, A. J. Influence of Civil Engineering Projects on Water Quality in Deltaic Regions. NBS Inorg. Sem. Series, Nov. 29, 1978.

78. Burnham, A. K., G. V. Calder, J. S. Fritz, G. A. Junk, H. J. Svec, and R. Willis. Anal. Chem., 44:139, 1972.

79. Morrison, G. H., and H. Freiser. Solvent Extraction in Analytical Chemistry. John Wiley and Sons, 1957. p. 62.

80. LaFleur, P. D., and W. P. Reed. Report of Investigation-Research Material 50 - Albacore Tuna.

81. Dokiya, Y., M. Taguchi, S. Toda, and K. Fuwa. Anal. Chem., 50:533, 1978.

82. Cappon, C. J., and J. C. Smith. Anal. Chem., 49:365, 1976.

83. Rains, T. C. Report of Analysis, NBS, 1978.

84. Westöö, G. Acta Chem. Scand., 20:213, 1966.

85. Westöö, G. Science, 181:567, 1973.

86. Bache, C. A., W. H. Gutenmank, and D. J. Lisk. Science, 172:951, 1971.

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